Appendix XI. Ecological Effects Characterization

a. Toxicity to Terrestrial Animals

I. Birds and Reptiles, Acute and Subacute

An acute oral toxicity study using the technical grade of the active ingredient (TGAI) is required to establish the toxicity of atrazine to birds. The preferred test species is either mallard duck (a waterfowl) or bobwhite quail (an upland gamebird). Results of this test are tabulated below.

Avian Acute Oral Toxicity

Surrogate Species	% ai	LD50 (mg/kg) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification[1]
Northern bobwhite quail (*Colinus virginianus*) 14-day old chicks; 8-day test	Tech.	940 slope 3.836	Slightly toxic	00024721 Fink 1976	Core
Mallard Duck (*Anas platyrhynchos*) 6-months old; 14-day test	76 % 80 WP	> 2,000 slope none	Practically non-toxic	00160000 Hudson, Tucker & Haegle 1984	Supplemental (only 3 birds) (formulation)
Ring-necked Pheasant (*Phasianus colchicus*) 3-months old; 14-day test	76 % 80 WP	> 2,000 slope none	Practically non-toxic	00160000 Hudson, Tucker & Haegle 1984	Supplemental (formulation)
Japanese Quail (*Coturnix c. japonica*) 50-60 days old; 14-day test	Tech.	4,237 slope > 6	Practically non-toxic	00024722 Sachsse and Ullman 1974	Supplemental (species not native)

[1] Core (study satisfies guideline). Supplemental (study is scientifically sound, but does not satisfy guideline)

Since the lowest LD_{50} (940 mg/kg) is in the range of 501 to 2,000 mg/kg, atrazine is categorized as slightly toxic to avian species on an acute oral basis. According to Hudson *et al*. (1984), signs of intoxication in mallards first appeared 1 hour after treatment and persisted up to 11 days. In pheasants, remission of signs of intoxication occurred by 5 days after treatment. Signs of intoxication included weakness, hyper-excitability, ataxia, tremors; weight loss occurred in mallards. The guideline requirement (71-1) is fulfilled (MRID 00024721).

Degradates: The major atrazine degradate, hydroxyatrazine, forms a large percentage of the recoverable pesticide in various compartments of the environment. Minor atrazine degradates include deethylatrazine, deisopropylatrazine and diaminochlorotriazine. Acute mammalian LD_{50} values available for deethylatrazine and deisopropylatrazine are both more toxic than the parent atrazine. Therefore, a special (70-1) acute oral test with the upland gamebird (preferably northern bobwhite) are required to address the concern for these three degradates. The requirement (70-1) has not been fulfilled.

Two subacute dietary studies using the TGAI are required to establish the toxicity of atrazine to birds. The preferred test species are mallard duck and bobwhite quail. Results of these tests are tabulated below.

Avian Subacute Dietary Toxicity

Surrogate Species	% ai	5-Day LC50 (ppm)[1]	Toxicity Category	MRID No. Author/Year	Study Classification
Northern bobwhite (*Colinus virginianus*) 9-days old chicks	99.0	> 5,000 (no mortality)	Practically non-toxic	00022923 Hill *et al*. 1975	Core
Northern bobwhite (*Colinus virginianus*) young adults	Tech.	> 10,000	Practically non-toxic	unknown - Gulf South Gough & Shellenberger 1972	Supplemental (Adult birds & no raw data)
Ring-necked pheasant (*Phasianus colchicus*) 10-days old chicks	99.0	> 5,000 (no mortality)	Practically non-toxic	00022923 Hill *et al*. 1975	Core
Japanese Quail (*Coturnix c. japonica*) 7-days old chicks	99.0	> 5,000 (7 % mortality at 5,000 ppm)	Practically non-toxic	00022923 Hill *et al*. 1975	Supplemental (species not native)
Mallard duck (*Anas platyrhynchos*) 10-days old ducklings	99.0	> 5,000 (30 % mortality at 5,000 ppm)	Practically non-toxic	00022923 Hill *et al*. 1975	Core

[1] Test organisms observed an additional three days while on untreated feed.

Since the LC_{50} values are greater than 5,000 ppm, atrazine is categorized as practically non-toxic to avian species on a subacute dietary basis. The time to death was Day 3 for the one Japanese quail and Day 5 for three mallard ducks (J. Spann at Patuxent Wildlife Center, 1999, personal communication). The guideline requirement (71-2) is fulfilled (MRID 00022923).

Subacute dietary studies using a typical end-use product (TEP) may be required on a case-by-case basis to establish the toxicity of atrazine formulations to birds. The preferred test species are mallard duck and bobwhite quail. Results of these tests are tabulated below.

Formulation Avian Subacute Dietary Toxicity

Surrogate Species	% ai Form	5-Day LC50 (ppm ai)[1] Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification
Northern bobwhite (*Colinus virginianus*) (6-weeks old)	76 80 WP	5,760 slope 3.252	Practically non-toxic	00059214 Beliles & Scott 1965	Supplemental (birds too old)
Mallard duck (*Anas platyrhynchos*)	76 80 WP	19,560 slope 1.807	Practically non-toxic	00059214 Beliles & Scott 1965	Core for 80W formulation

[1] Test organisms observed an additional three days while on untreated feed.

Since the LC50 values are greater than 5,000 ppm, atrazine is categorized as practically non-toxic to avian species on a subacute dietary basis for the 80W formulation. In the mallard study, a highly noticeable weight loss and emaciated birds were found at all test levels (1,000 to 32,000). No guideline requirement (71-2) is required for atrazine formulations.

Degradates: The major atrazine degradate, hydroxyatrazine, forms a large percentage of the recoverable pesticide in various environmental compartments. Acute mammalian LD_{50} values

available for deethylatrazine and deisopropylatrazine, minor degradates, are both more toxic than the parent atrazine. Special (70-2) oral dietary tests for these degradates with waterfowl (preferably mallard duck) and upland gamebird (preferably northern bobwhite) are reserved pending the results of acute oral toxicity tests on these degradates. The requirement (70-2) is reserved.

ii. Birds and Reptiles, Chronic

Avian reproduction studies using the TGAI are required for atrazine, because the following conditions are met: (1) birds may be subject to repeated or continuous exposure to the pesticide, especially preceding or during the breeding season, (2) the pesticide is stable in the environment to the extent that potentially toxic amounts may persist in animal feed, (3) the pesticide is stored or accumulated in plant or animal tissues, and/or, (4) information derived from mammalian reproduction studies indicates reproduction in terrestrial vertebrates may be adversely affected by the anticipated use of the product. The preferred test species are mallard duck and bobwhite quail. Results of these tests are tabulated below.

Avian Reproduction

Surrogate Species/ Study Duration	% ai	NOEC/LOEC (ppm ai)		Statistically sign. (p=0.05) LOEC Endpoints	MRID No. Author/Year	Study Classification
Northern bobwhite (*Colinus virginianus*) 20 weeks	97.1	NOAEC LOAEC	225 675	29 % red. in egg production 67 % incr. in defective eggs 27 % red. in embryo viability 6-13 % red. in hatchling body wt. 10-16 % red. in 14-day old body wt. 8.2 % red. in 14-day old body wt. (after recovery period)	42547102 Pedersen & DuCharme 1992	Core
Mallard duck (*Anas platyrhynchos*) 20 weeks	97.1	NOAEC LOAEC	225 675	49 % red. in egg production 61 % red. in egg hatchability 12-17 % red. in food consumption	42547101 Pedersen & DuCharme 1992	Core

In the bobwhite study, the reproductive endpoints were measured after a 3-week recovery period. A statistically significant effect during the recovery period was a 700 percent increase in the number of defective eggs at 675 ppm compared to controls; the number of defective eggs was consistent with the number of defective eggs during the treatment period at 675 ppm. Bobwhite and mallard tests show similar toxic effects on reduced egg production and embryo viability/hatchability at 675 ppm. In an 8-day LC_{50} test with adult Japanese quail, the quail fed atrazine had reduced food consumption, lost body weight and egg production stopped after 3 days of exposure (Sachsse and Ullman, 1975; MRID 00024723). The guideline requirement (71-4) is fulfilled for both avian species (MRID 42547101, 42547102).

Following the observation of sex changes from male to female alligators in Lake Apopka, Florida, Crain *et al*. (1997) treated alligator eggs with five environmental contaminants including two herbicides for endocrine alterations in juveniles. Atrazine (150, 1,400 and 14,000 ppb) induced gonadal-adrenal mesonephros (GAM) aromatase activity in male hatchling alligators

that is uncharacteristic of both males and females. However, the results of the atrazine study did not reflect the sexual effects observed in the alligators from Lake Apopka.

Degradates: The major atrazine degradate, hydroxyatrazine, forms a large percentage of the recoverable pesticide in various environmental compartments. Minor degradates were deethylatrazine, deisopropylatrazine and diaminochloro-s-triazine. Subchronic mammalian toxicity values available for deethylatrazine, deisopropylatrazine, hydroxyatrazine and diaminochlorotriazine indicate greater or equal toxicity compared to the parent atrazine in 10-day pregnancy tests, 13-week dog dietary tests, 1-year dog dietary tests and 2-year carcinogenicity tests. The requirement for avian reproductive tests with degradates are reserved pending the acute oral and dietary test results. The requirement (70-4) for degradates is reserved.

iii. Mammals, Acute

Wild mammal testing is required on a case-by-case basis, depending on the results of lower tier laboratory mammalian studies, intended use pattern and pertinent environmental fate characteristics. In most cases, rat or mouse toxicity values obtained from the Agency's Health Effects Division (HED) substitute for wild mammal testing. The acute toxicity values cited in HED's oneliners are reported below.

Mammalian Toxicity

Surrogate Species	% ai	Test Type	Toxicity Value	Affected Endpoints	MRID No*./ Author
Laboratory mouse (*Mus musculus*)	??	Acute oral	1,750 mg/kg	LD50 (mortality)	??? Weed Sci. Assoc. 1967
Laboratory rat (*Rattus norvegicus*)	Tech.	Acute oral	1,869 mg/kg	LD50 (mortality)	00230303 Ciba-Geigy 1975
Laboratory rat (*Rattus norvegicus*)	Tech.	Acute oral	2,030 mg/kg	LD50 (mortality)	00231466 Instituto di Ricerche
Laboratory rat (*Rattus norvegicus*)	95	Acute oral	2,850 mg/kg	LD50 (mortality)	00027097 Consultox Lab. Ltd.
Laboratory rat (*Rattus norvegius*)	??	Acute oral	3,080 mg/kg	LD50 (mortality)	??? Weed Sci. Assoc. 1967
Laboratory mouse *(Mus musculus)*	Tech.	Acute oral	3,992 mg/kg	LD50 (mortality)	00230303 Ciba-Geigy 1977
FORMULATIONS:					
Laboratory rat (*Rattus norvegicus*)	85.5	Acute oral - female male	1,180 mg/kg 1,317 mg/kg	LD50 (mortality)	00249196 Stillmeadow Inc. 1980
Laboratory rat (*Rattus norvegicus*)	85.5 90 W	Acute oral	1,440 mg/kg	LD50 (mortality)	00000527 Industry Bio-Test 1971
Laboratory rat (*Rattus norvegicus*)	85.5	Acute oral	1,992 mg/kg	LD50 (mortality)	00000847 Hill Top Research, Inc.

Mammalian Toxicity

Surrogate Species	% ai	Test Type	Toxicity Value	Affected Endpoints	MRID No*./ Author
Laboratory rat (*Rattus norvegicus*)	76 80 WP	Acute oral	>1,520 mg/kg <1,900 mg/kg	LD50 (mortality)	00046159 WIL Res. Lab. 1978
Laboratory rat (*Rattus norvegicus*)	76	Acute oral - male	2,147 mg/kg	LD50 (mortality)	00240852 Industrial Bio-Test 1974
Laboratory rat (*Rattus norvegicus*)	Atratol 8P	Acute oral	3,100 mg/kg (as product)	LD50 (mortality)	00234490 Food & Drug Res. 1977
Laboratory rat (*Rattus norvegicus*)	76 80 W	Acute oral	3,876 mg/kg	LD50 (mortality)	00230305 Industrial Bio-Test 1965
Laboratory rat (*Rattus norvegicus*)	51.0	Acute oral - female male	546 mg/kg 729 mg/kg	LD50 (mortality)	00245364 Food & Drug Res. Lab.
Laboratory rat (*Rattus norvegicus*)	44.3 Aatrex	Acute oral - female male	2,437 mg/kg 2,038 mg/kg	LD50 (mortality)	00000519 Not listed
Laboratory rat (*Rattus norvegicus*)	43 Flowable	Acute oral	830 mg/kg	LD50 (mortality)	00000522 Not listed
Laboratory rat (*Rattus norvegicus*)	42.0	Acute oral	811 mg/kg	LD50 (mortality)	00002041 Bio/Dynamics Inc. 1976
Laboratory rat (*Rattus norvegicus*)	40.8	Acute oral - female male	224 mg/kg 738 mg/kg	LD50 (mortality)	00242662 Raltech Sci. Serv. 1980
Laboratory rat (*Rattus norvegicus*)	40.8	Acute oral - female male	432 mg/kg 690 mg/kg	LD50 (mortality)	00000537 WIL Res. Lab. 1978
Laboratory rat (*Rattus norvegicus*)	40.8	Acute oral - female male	439 mg/kg 769 mg/kg	LD50 (mortality)	00246393 Toxigenics, Inc 1981
Laboratory rat (*Rattus norvegicus*)	40.8	Acute oral - female male	> 694 mg/kg 775 mg/kg	LD50 (mortality)	00243485 Cosmopolitan Safety Evalutation, Inc 1980
Laboratory rat (*Rattus norvegicus*)	40.8	Acute oral	1,306 mg/kg	LD50 (mortality)	00253726 Bio/Dynamics Inc. 1984
Laboratory rat (*Rattus norvegicus*)	40.8 Atrazine 4L	Acute oral - female male	1,918 mg/kg 1,705 mg/kg	LD50 (mortality)	00241725 Cosmopolitan Safety Evaluation, Inc

* ??? indicates that MRID no. is pending

An analysis of the results indicate that atrazine and its formulations range from 224 to 3,992 mg/kg which categorized atrazine as moderately to slightly toxic to small mammals on an acute oral basis. Initial symptoms, piloerection and decreased activity, were reported as early as 30 minutes posttreatment. Other signs of toxicity include salivation, lacrimation, muscular weakness, tremor ataxia, diarrhea, adrenal degradation, congested lungs, and degeneration of kidneys and adrenal glands. Matching toxicity values for males and females in most cases (i.e., 7 out of 9 studies) indicate that females are more sensitive to atrazine than male rats. Atrazine does not appear to be dermally toxic to adult rats and rabbits; dermal LD50 values are greater than 2,000 mg/kg. Atrazine generally causes corneal opacity which clears by day 7. The need for mammalian acute toxicity is fulfilled.

Degradates: The major atrazine degradate, hydroxyatrazine, forms a large percentage of the recoverable pesticide in various compartments of the environment. Therefore, a special (70-3) acute oral test with small mammals is required to address degradate concerns.

Degradate Mammalian Acute Oral Toxicity

Surrogate Species/ Study Duration	% ai	Test Type	Toxicity Value	Affected Endpoints	MRID No.
Laboratory rat (*Rattus norvegicus*)	95.7 % Deethylatrazine (G-30033)	Acute oral - female male	668 mg/kg 1,891 mg/kg	LD50 (mortality) signs within 30 minutes died within 24-48 hours	43012302
Laboratory rat (*Rattus norvegicus*)	Tech. Deisopropylatrazine (G-28279)	Acute oral - female male	810 mg/kg 2,290 mg/kg	LD50 (mortality) signs within 0.5-48 hours died within 6-48 hours	43012301

Acute mammalian oral toxicity data are available for two degradates, deethylatrazine and deisopropylatrazine. The female LD_{50} values are more toxic to laboratory rats than technical grade values for the parent pesticide, atrazine. These degradates have LD_{50} values between 501 and 2,000 mg/kg which indicates that these degradates are slight toxicity orally. As with atrazine, the female toxicity values for the degradates indicate greater toxicity than for male rats. The requirement (71-3) for these two degradates are fulfilled, but the requirement (70-3) has not been fulfilled for the major degradate, hydroxyatrazine.

iv. Mammals, Chronic

Wild mammal reproduction testing is required on a case-by-case basis, depending on the results of lower tier laboratory mammalian studies, intended use pattern and pertinent environmental fate characteristics. Atrazine is persistent and initial residue levels exceed acute toxicity values. Usually mammalian chronic data are rat and/or mouse toxicity values are obtained from the Agency's Health Effects Division (HED) and substitute for wild mammalian testing. HED reproductive and systematic toxicity values are reported below.

Mammalian Subchronic and Reproduction Toxicity

Surrogate Species/ Study Duration	% ai	NOAEL/LOAEL (ppm ai)		Statistically sign. (p=0.05) LOEC Endpoints	MRID No. Author/Year	Study Classification
Laboratory Rat (*Rattus norvegicus*) 2-Generation Dietary	Tech.	NOAEL LOAEL NOAEL LOAEL	50 500 10 50	red. adult body weight and red. adult food consumption red. pup body weight in second generation	40431303 Ciba-Geigy 1987	Minimum
Laboratory Rat (*Rattus norvegicus*) 2-Yr Carcinogenicity	98.9	NOAEL LOAEL NOAEL LOAEL	10 70 70 500	incr. carcinomas for females (adenomas & fibroadenomas) red. mean adult body weight	00158930 American Biogenics Corp. 1986	Minimum
Laboratory Rat (*Rattus norvegicus*) 2-Yr Carcinogenicity	97	NOAEL LOAEL	70 400	red. adult body weight gain	42204401 Hazleton Lab. 1992	Minimum

Mammalian Subchronic and Reproduction Toxicity

Surrogate Species/ Study Duration	% ai	NOAEL/LOAEL (ppm ai)	Statistically sign. (p=0.05) LOEC Endpoints	MRID No. Author/Year	Study Classification
Laboratory Rat (*Rattus norvegicus*) Fed for 14 days	97.4	NOAEL 100 LOAEL 200	reduced estrogen levels	41570901 Hazelton Lab. 1990	Supplementary
Laboratory rat (*Rattus norvegicus*) Dosed on Days 6 - 15	97.4	NOAEL 100 LOAEL 500	increased fused sternebrae 1 & 2	43012308 Ciba-Geigy 1992	Guideline
Laboratory rat (*Rattus norvegicus*) Dosed on Days 6 - 15	95.7	NOAEL 100 LOAEL 500 NOAEL 500 LOAEL 2,000	17 % red. adult body weight gain incr. in fused sternebrae 1 & 2 incr. poor ossification of fifth toe	43013209 Ciba-Geigy 1992	Guideline
Laboratory rat (*Rattus norvegicus*) Dosed on Days 6-15 ?	96.7	NOAEL 200 LOAEL 1,400	red. body weight gain delayed ossification	40566301 Ciba-Geigy 1984	Minimum
Dog – Beagle (*Canis* sp.) 13-Week Feeding	Tech.	NOAEL < 200 LOAEL 200	red. body weight in males	00163339 WARF Institute 1997	Supplementary
Dog – Beagle (*Canis* sp.) 1-Year Feeding	97	NOAEL 150 LOAEL 1,000	increases in deaths, cachexia, ascite decr. body weight & food consumption irregular heart beat, incr. heart rate, incr. cardiac lesions	40431301 41293800 Ciba-Geigy 1987	Minimum
New Zealand Rabbit (L*epis* sp.)	96.3	NOAEL 33 LOAEL 165 NOAEL 165 LOAEL 2,475	red. body weight gain and red. food consumption. incr. resorptions, red. fetal body weights and delayed ossification of appendages	00143008 40566301 Ciba-Geigy 1984	Supplemental Minimum
Laboratory mice (*Mus musculus*) 22-Month Oncology	batch 841802	NOAEL 300 LOAEL 1,500	23.5 % red. male body weight 11 % red. female body weight incr. incidence of cardiac thrombi in females	40431302 Ciba-Geigy 1987	Guideline

The above mammalian chronic studies provide adequate toxicity data on chronic and reproductive effects. HED has concluded there is evidence that atrazine is associated with endocrine disruption. Direct measurements of norepinephrine, dopamine, and GnRH, and of serum hormones such as certain steroid hormones and luteinizing hormone, as well as changes in estrous cycling and histomorphic changes in hormone responsive tissues, indicate neuroendocrine disruption. The need for chronic mammalian toxicity data is fulfilled.

Degradates: The major atrazine degradate, 2-hydroxyatrazine, forms a large percentage of the recoverable pesticide in various environmental compartments. Several subchronic and chronic toxicity studies for atrazine degradates and/or metabolites are summarized in the table below for deethylatrazine, deisopropylatrazine, diaminochlorotriazine and hydroxyatrazine.

Degradate Mammalian Subchronic and Reproduction Toxicity

Surrogate Species/ Study Duration	% ai	NOAEL/LOAEL (ppm ai)	Statistically sign. (p=0.05) LOAEL Endpoints	MRID No. Author/Year	Study Classification
Laboratory rat (*Rattus norvegicus*) Fed for 14 days	assumed to be 98.2 Diaminochlorotriazine (G-28273)	NOAEL < 100 LOAEL 100 200	red. LH and prolactin levels red. estrogen, LH, prolactin and progesterone	41570901 Hazleton Lab. 1990	Supplemental
Laboratory rat (*Rattus norvegicus*) Diet for 13 weeks	95.7 Deethylatrazine (G-30033)	NOAEL 50 LOAEL 500	red. in female body weight red. in food efficiency for male and female rats	43012306 Ciba-Geigy 1991	Acceptable-Guideline
Laboratory rat (*Rattus norvegicus*) Diet for 13 weeks	96.7 Deisopropyltriazine (G-28279)	NOAEL 50 LOAEL 500	red. in body weights and red. body weight gains in males and females	43012305 Ciba-Geigy 1992	Core-Guideline
Laboratory rat (*Rattus norvegicus*) Diet for 13 weeks	98.2 Diaminochlorotriazine (G-28273)	NOAEL 10 LOAEL 100 NOAEL 100 LOAEL 250	Estrous cycle effects in female rats red. body weight gain in males and females Week 12	43012307 Ciba-Geigy 1991	Core-Guideline
Laboratory rat (*Rattus norvegicus*) Diet for 13 weeks	97.1 Hydroxyatrazine (G-34048)	NOAEL 100 LOAEL 300	renal effects - high urine output with low S.G. red. hematopoietic parameters in both sexes	41293501 Ciba-Geigy 1989	Minimum
Dog – Beagle (*Canis* sp.) 13-Week Feeding	95.7 Deethylatrazine (G-30033)	NOAEL 100 LOAEL 1,000	red. body weight & weight gain in males and females; red. heart to brain weight; normocytic/normochromic anemia, paroxysmal atrial fibrillation and right atrial wall hemorrhagic inflammation with angiomatous hyperplasia	43012304 Ciba-Geigy 1992	Core-Minimum
Dog -- Beagle (*Canis* sp.) 14-Week Feeding	96.7 Deisopropylatrazine (G-28279)	NOAEL 100 LOAEL 500	red. body weight, weight gain and food consumption in females red. organ to brain weight for heart, testes, prostrate glands in males	43012303 Ciba-Geigy 1992	Core-Minimum
Dog – Beagle (Canis sp.) 1-Year Feeding	98.7 Diaminochlorotriazine (G-28273)	NOAEL 5 LOAEL 100	1 of 8 females had tremors	41392401 Ciba-Geigy 1990	Minimum
Laboratory rat (*Rattus norvegicus*) Dosed on Days 6-15	95.7 Deethylatrazine (G-30033)	NOAEC 5 LOAEC 25 Development: NOAEL 25 LOAEC 100	red. body weight; weight gain and food consump. Fused sternebrae 1 & 2 Poor ossification of digit 5	43013209 Ciba-Geigy 1992	Acceptable-Guideline
Laboratory rat (*Rattus norvegicus*) Dosed on Days 6-15	97.4 Deisopropylatrazine (G-28279)	NOAEL 5 LOAEL 25 Development: NOAEL 5 LOAEL 25	red. maternal body weight, weight gain & food consumption fused sternebrae 1 and 2. poor ossification at 100 ppm	43012308 Ciba-Geigy 1992	Core - Guideline

Degradate Mammalian Subchronic and Reproduction Toxicity

Surrogate Species/ Study Duration	% ai	NOAEL/LOAEL (ppm ai)	Statistically sign. (p=0.05) LOAEL Endpoints	MRID No. Author/Year	Study Classification
Laboratory rat (*Rattus norvegicus*) Dosed on Days 6-15	98.2 Diaminochlorotriazine (G-28273)	NOAEL 500 LOAEL 3000 Development: NOAEL 50 LOAEC 500	red. body weight gain and food consumption incr. resorption of embryos incr. unossified bones	41392402 Ciba-Geigy 1989	Minimum
Laboratory rat (*Rattus norvegicus*) Dosed on Days 6-15	97.1 Hydroxyatrazine (G-34048)	NOAEL 500 LOAEL 2,500 Development: NOAEL 500 LOAEL 2500	decr. mother's food consumption red. young body weight incr. delay in ossification of skull bones	41065202 42873702 Ciba-Geigy 1989	Minimum
Laboratory rat (*Rattus norvegicus*) 2-yr Carcinogenicity	97.1 Hydroxyatrazine (G-34048)	NOAEL 10 LOAEL 25	incr. in accumulation of interstitial matrix in the kidney in females only	43532001 Ciba-Geigy 1995	Reserved
Laboratory rat (*Rattus norvegicus*) 2-yr Carcinogenicity	97.1 Hydroxyatrazine (G-34048)	NOAEL 25 LOAEL 200	incr. in urinary tract effects in both sexes	42662901 Ciba-Geigy 1993	Supplemental

Comparison of various subchronic and chronic toxicity levels for the following degradates (deethylatrazine, deisopropylatrazine, diaminochlorotriazine and hydroxyatrazine) with atrazine data suggest that the toxicity of these degradates are equal to or slightly more toxic to laboratory rats and beagles than atrazine. The degradate studies typically show similar types of toxic effects seen in atrazine tests. The mammalian chronic studies provide adequate data on chronic and reproductive effects of degradates.

v. Insects

A honey bee acute contact study using the TGAI is required for atrazine because its wide-spread use on corn and other crops that need insect pollination will result in honey bee exposure. Results of this test are tabulated below.

Nontarget Insect Acute Contact Toxicity

Surrogate Species	% ai	LD50 (Fg/bee)	Toxicity Category	MRID No. Author/Year	Study Classification
Honey bee *(Apis mellifera)*	Tech.	96.69 (4.79% dead)	relatively non-toxic	00036935 Atkins *et al*. 1975	Core

Test results indicate that atrazine is categorized as relatively non-toxic to bees on an acute contact basis. The guideline (141-1) is fulfilled (MRID 00036935).

A honey bee toxicity of residues on foliage study using the typical end-use product is not required for atrazine because the acute contact honey bee LD50 is greater than 0.11 Fg/bee. The guideline requirement (141-2) is fulfilled (MRID 00036935).

vi. Terrestrial Field Testing

No field tests have been required, because atrazine shows low toxicity to birds, mammals and insects.

b. Toxicity to Freshwater Animals

I. Freshwater Fish and Amphibia, Acute

Two freshwater fish toxicity studies using the TGAI are required to establish the toxicity of atrazine to fish. The preferred test species are rainbow trout (a coldwater fish) and bluegill sunfish (a warmwater fish). Results of these tests are tabulated below.

Freshwater Fish Acute Toxicity

Surrogate Species/ Static or Flow-through test	% a.i.	96-hour LC50 (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No.* Author/Year	Study Classification
Rainbow trout (*Oncorhynchus mykiss*) Static test	98.8	5,300 (nominal) slope - 2.723	moderately toxic	00024716 Beliles & Scott 1965	Core
Brook trout (*Salvelinus tontinalis*) Flow-through test	94	6,300 4,900 (8-day test) not specified	moderately toxic	00024377 Macek *et al*. 1976	Supplemental (52-gram fish & no raw data)
Fish from the Nile River *Chrysichthyes auratus* Static-renewal - daily 150 mg/L $CaCO_3$; 22EC	96	6,370 (not specified)	moderately toxic	45202911 Hussein, El-Nasser & Ahmed 1996	Supplemental (non-native sp.; 26-gram fish; no raw data)
Bluegill sunfish (*Lepomis macrochirus*) Flow-through test	94	> 8,000 6,700 (7-day test) (not specified)	moderately toxic	00024377 Macek *et al*. 1976	Supplemental (6.5-gram fish & no raw data)
Tilapia 38 grams (*Oreochromis niloticus*) Static-renewal - daily 150 mg/L $CaCO_3$: 22EC	96	9,370 (not specified)	moderately toxic	45202911 Hussein, El-Nasser & Ahmed 1996	Supplemental (non-native sp.; 38-gram fish; no raw data)
Fathead minnow (*Pimephales promelas*) 24-Hour renewal test	94	15,000 (nominal) 15,000 (5-day test)	slightly toxic	00024377 Macek *et al*. 1976	Supplemental (no raw data)
Carp (*Cyprinus carpio*) Semi-static test	93.7	18,800 (nominal) slope not reported	slightly toxic	45202913 Neskovic *et al*. 1993	Supplemental (no raw data)
Fathead minnow juvenile (*Pimephales promelas*) Flow-through test; 52 mg/L $CaCO_3$	97.1	20,000 (measured) Slope - 6.889	slightly toxic	42547103 Dionne 1992	Core
Bluegill sunfish (*Lepomis macrochirus*) Static test	98.8	24,000 (nominal) no slope	slightly toxic	00024717 Beliles & Scott 1965	Core

Freshwater Fish Acute Toxicity

Surrogate Species/ Static or Flow-through test	% a.i.	96-hour LC50 (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No.* Author/Year	Study Classification
Brown trout (*Salmo trutta*) 1.9 gr. Static-Renewal - daily pH 6; 10EC; 11 mg/L $CaCO_3$	??	27,000 (nominal)	slightly toxic	45202909 Grande, Anderson & Berge 1994	Supplemental (no raw data; slight aeration & purity unknown)
Zebrafish (*Brachydanio rerio*)	??	37,000 (not specified)	slightly toxic	??? Korte & Greim 1981	Supplemental (article unavailable)
Bluegill sunfish (*Lepomis macrochirus*) Static test	100	57,000 (nominal)	slightly toxic	00147125 Buccafusco 1976	Core
Goldfish (*Carassius auratus*) Static test	98.8	60,000 (nominal) Slope - 2.695	slightly toxic	00024718 Beliles & Scott 1965	Supplemental (not an acceptable species)

* ??? indicates MRID no. is pending

The lowest fish LC_{50} value falls in the range of > 1 - 10 ppm, hence atrazine is categorized as moderately toxic to freshwater fish on an acute basis. The guideline requirement (72-1) is fulfilled (MRID 00024716, 00024717, 000147125).

The following table presents fish toxicity data for formulated products.

Freshwater Fish and Amphibian Acute Toxicity

Surrogate Species/ Flow-through or Static	% ai formul.	96-hour LC50 (ppb) (measured/nominal)	Toxicity Category	MRID No. Author/Year	Study Classification
Black Bass - fry (*Micropterus salmoides*) Static test; 20EC 78 mg/L hardness	80 80 W	12,600 (nominal) slope - 5.86	slightly toxic	45227717 R. O. Jones 1962	Supplemental (48-hours; limited raw data)
Channel Catfish yolk sac (*Ictalurus punctatus*) Static test; 23.3-25.8EC 78 mg/L hardness	80 80 W	16,000 (nominal) slope - 3.36	sightly toxic	45227717 R. O. Jones 1962	Supplemental (limited raw data)
Bluegill Sunfish fry (*Lepomis macrochirus*) Static test; 25-27EC 78 mg/L hardness	80 80 W	20,000 (nominal) no slope	slightly toxic	45227717 R. O. Jones 1962	Supplemental (limited raw data)
American Toad - larvae (*Bufo americanus*) Flow-through test	40.8 4L	10,700 late stage 26,500 early stage (nominal)	slightly toxic	45202910 Howe *et al*. 1998	Supplemental (no raw data)
Northern Leopard Frog larvae (*Rana pipiens*) Flow-through test	40.8 4L	14,500 late stage 47,600 early stage (nominal)	slightly toxic	45202910 Howe *et al*. 1998	Supplemental (no raw data)
Coho Salmon (*Oncorhynchus kisutch*) Renewal daily; 144 hr	40.8* AAtrex Liquid	> 18,000 25 % mortality (measured)	slightly toxic	45205107 Lorz *et al*. 1979	Supplemental (no LC_{50} value & 12-17 months old)

Freshwater Fish and Amphibian Acute Toxicity

Surrogate Species/ Flow-through or Static	% ai formul.	96-hour LC50 (ppb) (measured/nominal)	Toxicity Category	MRID No. Author/Year	Study Classification
Rainbow trout (*Onchorhynchus mykiss*) Flow-through test	40.8 4L	20,500 (nominal)	slightly toxic	45202910 Howe *et al*. 1998	Supplemental (no raw data)
Channel Catfish (*Ictalurus punctatus*) Flow-through test	40.8 4L	23,800 (nominal)	slightly toxic	45202910 Howe *et al*. 1998	Supplemental (no raw data)
Rainbow trout (*Oncorhynchus mykiss*) Static test	43 Liquid	24,000 (unknown)	slightly toxic	40098001 Mayer & Ellersieck 1986	Supplemental (no raw data)
Bluegill sunfish (*Lepomis macrochirus*) Static test	43 Liquid	42,000 (unknown)	slightly toxic	40098001 Mayer & Ellersieck 1986	Supplemental (no raw data)

* Percent a.i. assumed based on description as a liquid formulation, AAtrex.

All toxicity values for the atrazine formulation are > 10 and 100 ppm, therefore this atrazine product is classified as slightly toxic.

Degradates: The major atrazine degradate, hydroxyatrazine, forms a large percentage of the recoverable pesticide in aquatic environmental compartments. Therefore, acute fish testing with bluegill and rainbow trout are required to address degradate concerns. The requirement for special degradate tests (72-1) has not been fulfilled.

ii. Freshwater Fish, Chronic

A freshwater fish early life-stage test using the TGAI is required for atrazine because the end-use product is expected to be transported to water from the intended use site, and the following conditions are met: the pesticide is intended for use such that its presence in water is likely to be continuous and recurrent; an aquatic acute EC50 is less than 1 mg/L (i.e., *Chironomus tentans* LC_{50} 0.72 ppm); and the pesticide is persistent in water (*i.e.*, half-life greater than 4 days). The preferred test species is rainbow trout.

Freshwater Fish Early Life Stage Toxicity

Surrogate Species/ Study Duration/ Flow-through or Static Renewal	% ai	NOAEC/LOAEC Fg/L (ppb) (measured or nominal)	Statistically sign. (p=0.05) Endpoints Affected	MRID No. Author/Year	Study Classification
Rainbow trout (*Oncorhynchus mykiss*) 86 days, flow-through 50 mg/L $CaCO_3$	Tech.	NOAEC 410 LOAEC 1,100 (measured)	sign. delays in hatching @ 1,100 and 3,800 Fg/L sign. red. wet wt. at 30 & 58 days @ 1,100 & 3,800 Fg/L sign. red. dry wt. @ 3,800 Fg/L 58.8 % mortality @ 3,800 Fg/L at swim-up	45208304 Whale *et al*. 1994	Invalid (DMSO used as solvent, which aids in transport of chemicals across cell membranes)

Freshwater Fish Early Life Stage Toxicity

Surrogate Species/ Study Duration/ Flow-through or Static Renewal	% ai	NOAEC/LOAEC Fg/L (ppb) (measured or nominal)	Statistically sign. (p=0.05) Endpoints Affected	MRID No. Author/Year	Study Classification
Rainbow trout embryo-larvae (*Oncorhynchus mykiss*) 27 days; flow-through	80 WP	Hardness 50 mg/L: LC50 660 LC01 29 Slope 1.2 Hardness 200 mg/L: LC50 810 LC01 77 Slope 1.38	% normal survival 50/200 mg/L 19 Fg/L - 94 98 54 - 88 90 54 ** - 68 74 5,020 ** - 10 9 50,900 ** - 0 0	45202902 Birge, Black & Bruser 1979	Supplemental (short test; no raw data for statistical analyses)
Channel catfish embryo-larvae (*Ictalurus punctatus*) 8 days; flow-through	80 WP	Hardness 50 mg/L: LC50 220 Slope 0.977 Hardness 200 mg/L: LC50 230 Slope 0.84	highly teratogenic in all tests; no results for soft water 420 Fg/L - 16% terata 830 Fg/L - 47 % terata 46,700 Fg/L - 86 % terata	45202902 Birge, Black & Bruser 1979	Supplemental (short test; no raw data for statistical analyses)
Zebrafish (*Brachydanio rerio*) 35 Days; pH 8; 27±1EC Flow-through test Hardness 24 mg/L	98	NOAEC 300 LOAEC 1,300 (measured) 35-Day LC50 890 Slope 1.25	2 - 3 % sign. incr. in edema 45-62 % mortality	45202908 Gorge & Nagel 1990	Supplemental (no raw data)

In addition to survival of rainbow trout and catfish embryo-larvae, Birge *et al*. (1979) also reported that "Atrazine was highly teratogenic in all tests." The frequency of teratogenicity was reported for channel catfish in hard water and included in the table above; no data on frequency was reported for soft water or for rainbow trout. (MRID # 45202902). The guideline requirement (72-4) for a fish early life stage test is fulfilled by four fish life-cycle tests with rainbow trout, bluegill and fathead minnows (listed below).

Degradates: The major atrazine degradate, hydroxyatrazine, forms a large percentage of the recoverable pesticide in aquatic environmental compartments. Therefore, a special fish early life-stage test (72-4) is reserved to address degradate concerns, pending the results of acute fish tests.

A freshwater fish life-cycle test using the TGAI is required for atrazine because the end-use product is expected to be transported to water from the intended use site and studies of other organisms indicate that the reproductive physiology of fish may be affected. The preferred test species is fathead minnow. Results of four fish life-cycle tests are tabulated below.

Freshwater Fish Life-Cycle Toxicity

Surrogate Species/ Study Duration/ Flow-through or Static Renewal	% ai	NOAEC/LOAEC Fg/L (ppb) (measured or nominal)	Statistically sign. (p=0.05) Endpoints Affected	MRID No. Author/Year	Study Classification
Brook trout (*Salvelinus fontinalis*) 44 weeks, flow-through	94	NOAEC 65 LOAEC 120 (measured)	7.2 % red. mean length 16 % red. mean body weight	00024377 Macek *et al*. 1976	Core

Freshwater Fish Life-Cycle Toxicity

Surrogate Species/ Study Duration/ Flow-through or Static Renewal	% ai	NOAEC/LOAEC Fg/L (ppb) (measured or nominal)	Statistically sign. (p=0.05) Endpoints Affected	MRID No. Author/Year	Study Classification
Bluegill sunfish (*Lepomis macrochirus*) 6-18 months, flow-through	94	NOAEC 95 LOAEC 500 (measured)	LOAEC based on loss of equilibrium in a 28-day test conducted at the same lab.	00024377 Macek *et al*. 1976	Supplemental (Low survival in the controls)
Fathead minnow (*Pimephales promelas*) 39 weeks; flow-through	97.1	NOAEC < 150 LOAEC 150 (measured)	6.7 % red. in F_1 length 22 % red. in F_1 body wt. (sign. diff. from neg. control)	42547103 Dionne 1992	Supplemental (Failed to identify a NOAEC)
Fathead minnow (*Pimephales promelas*) 43 weeks, static-renewal	94	NOAEC 210 LOAEC 870 (measured)	LOAEC based on 25% mortality in a 96-hour test conducted at the same lab.	00024377 Macek *et al*. 1976	Supplemental (High mortality in control adults)

Moore and Waring (1998) report atrazine effects on reproductive endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr exposed to nominal concentrations of 0.5, 5, 10, and 20 Fg/L, which were collected and measured at the end of the test. The measured levels are reported as 0.04, 3.6, 6.0 and 14.0 Fg/L which are 8, 72, 60, and 70 percent of nominal, respectively. There appears to be uncertainty about the test concentrations, since the water samples were collected only after the test period and the authors concluded that atrazine in the water samples suffered rapid degradation as the result of an unavoidable delay in being analyzed. The guideline requirement (72-5) is fulfilled by the brook trout study (MRID 00024377).

Sublethal Effects:

Authors of several laboratory studies have reported statistically significant ($p < 0.05$) sublethal effects from atrazine on behavior (i.e.,mating behavior at $\geq$ **0.04 Fg/L** and increased preference for darkness at $\geq$ **5 Fg/L**), organs (i.e., kidney damage at **5 Fg/L** and reduced glutathione (GSH) in the liver at **50 Fg/L**), and tissues (i.e., increased muscle RNA/DNA at **10 Fg/L**) at low concentrations of atrazine which are found in surface waters. Sublethal effects at higher concentrations include gill damage at **500 Fg/L**, reduced alkaline phosphatase activity in the liver at **1,500 Fg/L**, and decreases in the number of red blood cells (RBC), hemoglobin and haematocrit levels compared to controls in two fish species at **3,000 Fg/L.** A Kansas pond field study shows reductions in bluegill dietary levels, numbers of species in diet, and reproduction resulting from indirect effects on pond vegetation following a single atrazine treatment at **20 Fg/L** (Kettle et al., 1987) (See paragraphs below).

Quantitative behavioral effects were found to be significant ($p < 0.0001$) in zebrafish (*Brachydanio rerio*) following 1-week exposures at **5 to 3125 Fg/L** atrazine (Steinberg *et al*., 1995). During pre-exposure conditions, about two-thirds of the swimming records were taken in the dark part of the aquarium. Fish exposed to atrazine for 1-week showed a pronounced preference ($p < 0.0001$) for the dark part of the aquarium compared to the control. Since no significant difference were found between the effects at the various test concentrations (5Fg/L: 79%; 25Fg/L: 85%; 125 Fg/L: 83%; 625 Fg/L: 81%; 3125 Fg/L: 81%), these changes in swimming behavior appears to be threshold effects. After 4 weeks at the above exposures, 15 to

24 percent more of the treated fish preferred dark habitats than did the controls. The authors concluded that atrazine probably has an effect on the sensory organs and the nervous system at atrazine concentrations commonly found in surface waters.
(MRID # 45204910).

Saglio and Trijase (1998) measured 5 behavioral activities in goldfish following 24-hour exposures to 0.5, 5 and 50 Fg/L atrazine. A number of behavioral measurements were statistically significant ($p < 0.05$) from controls, but in most instances the significance was inconsistent and failed to show a dose-related effect. The only behavioral effect showing a consistent, dose-related effect was reduction in grouping (i.e., significant at **5 Fg/L** (31% reduction) and 50 Fg/L (39% reduction). Other behaviors with statistically significant effects were surfacing at **5 Fg/L** (341% increase), burst swimming at 0.5 and **50** Fg/L (1.00 and 2.25 units, respectively, the controls showed no effect). Following the introduction of skin extract, **5 Fg/L** of atrazine significantly ($p < 0.05$) reduced sheltering (81%) and grouping (60%), but these effects showed no consistency with effects at 0.5 and 50 Fg/L. In goldfish tests with behavioral responses to subsequent atrazine-contaminated water, again showed statistically significant effects with respect to burst swimming at **0.5 Fg/L** (7.00 units), **5.0** Fg/L (3.75 units) and **50** Fg/L (4.00 units); the controls were 0.0 and 0.25 units. This study shows that a 24-hour exposure at **5 Fg/L** atrazine significantly affected aspects of swimming, positioning in water column, increased number of mouth openings at the surface, and social behaviors and did not produce drastic changes at **0.5 Fg/L**, except for a significant increase in number of burst swimming reactions. Burst swimming reactions are frequency observed in fish in response to stressful situations (MRID # 45202914).

Fischer-Scherl *et al*. (1991) reported acute and chronic atrazine-induced alterations in the rainbow trout kidneys affecting renal corpuscles, renal tubules, and renal interstitium. Although only minor changes were found at lower concentrations in chronic exposure, alterations of renal corpuscles are considerable, as comparisons to glomerulonephritis in man and mammals reveal. Additionally, the accumulation of cellular debris in Bowman's space affects glomerular filtration. **Renal Corpuscles:** Compared to control fish, chronic 28-day exposures at **5, 10 and 20 Fg/L** almost obliterated Bowman's space due to a proliferation of podocytes with their epithelial foot processes forming tight and intensive connections. The most conspicuous feature was the thickening of the glomerular basement membrane, with formation of so-called spikes. In some glomerula sub-endothelial humps, electron-dense deposits attached to glomerular basement membrane, have been detected. In some instances, moderate electron-dense material and membranous structures were deposited in Bowman's space. At higher chronic concentrations (**40 and 80 Fg/L**) renal corpuscles appeared hypercellular and enlarged due to a proliferation of podocytes and mesangial cells. Also, the amount of membrane-bound vesicles with varying electron-dense contents had increased in the urinary space of renal corpuscles. Fibrillar structures and fibrocytes were found around Bowman's capsule indicating beginning periglomerular fibrosis. Acute 96-hour exposures at **1.4 and 2.8 mg/L** caused a more pronounced obliteration of Bowman's space due to the proliferation of mesangial cells and more renal corpuscles were affected. Increasing amounts of cellular debris accumulated in Bowman's space. Simultaneously, epithelial cells of the parietal layer of Bowman's capsule displayed an

increased number of lysosomes and swollen mitochondria. Also, the number of glomerular endothelial cells exhibiting vacuolar degeneration increased.

Renal Tubules: Light microscopy shows minor alterations to renal tubules, but electron micrographs revel considerable changes. First, obvious alterations of tubules appeared at **10 Fg/L**. Basilar labyrinth was dilated and irregularly arranged. The mitochondria were electron-dense and showed club-shaped ends of circular structure. At **40 Fg/L**, part of the endoplasmic reticulum appeared foamy and fragments of endoplasmic reticulum were heavily distended; also mitochondria were involved in this process with their cristae mitochondriales being hardly discernible. At **80 Fg/L** in proximal and distal tubular epithelia lysis of the cytoplasm with formation of vacuoles and vesicles and condension of mitochondria was prominent. In many tubular epithelia, only remnants of the former parallel-arranged tubular system were present, mitochondria were swollen, lysosomal structures as well as a vacuolization of the cytoplasm were detectable. In proximal tubules, lysomes had increased in number and size. At acute exposures (**1,400 and 2,800 Fg/L**), tubular structural lesions similar to those described at **80 Fg/L** were present, but a distinctly higher number of renal tubules was affected. Extensive cytoplasmic vacuolization was evident and the parallel arrangement of the basilar labyrinth was completely lost, some mitochondria were dark and condensed. Tubules of the basilar labyrinth appeared foggy, partly involving mitochondria.

Renal Interstitium: Except for an increase in cells with mitotic figures at concentrations of 5, 10, 20 Fg/L, no conspicuous alterations in basic interstitial architecture could be detected. Beginning at **40 Fg/L**, a loosening of the hemopoietic tissue was evident. Cells, preumably macrophages, phagocytizing material had increased in number. In addition to these effects, sinusendothelial cells were severely damaged at a concentration of **80 Fg/L**. They separated from the basement membrane and exhibited numerous vesicular and lysosomal structures as well as swollen degenerating mitochondria. Alterations in renal interstitium were considerable at acute exposures with **1,400 and 2,800** Fg/L. Interstitial tissue was loosened and a state of spongiosus was indicated. Numerous macrophages were present. Nuclei of interstitial cells were pyknotic or karyorhectic, mitochondria were swollen and the cytoplasm displayed lytic areas. Cell boundaries in some parts of the interstitium were lost. Cell organelles were scarce, but lysosomal structures abundant. (MRID # 45202907)

Davies *et al*. (1994) exposed three fish species to 0.9, 3.0, 10, 50 and 340 Fg/L atrazine for a period of 10 days and measured effects on growth and properties of various tissues, such as blood, muscle and liver. Statistically significant ($p < 0.05$) effects occurred at levels as low as 0.9 and 3.0 Fg/L, but the results did not show consistent significant effects at levels less than **10 Fg/L** and in most cases failed to be dose-related. The most sensitive, consistent statistically significant effect was with *Galaxias maculatus* at **10 Fg/L** (i.e., 144% increase in muscle RNA/DNA levels, 145% increase at **50 Fg/L** and 141% increase at **340 Fg/L**); leucocrit level was significantly reduced 46% at **50 Fg/L**; and the DNA levels were significantly reduced 26%, 25% and 25% at **0.9, 3.0 and 10 Fg/L**, respectively, but yield no significant effect on DNA at 50 and 340 Fg/L. In *Pseudaphritis urvillii* consistent significant effects were found on glutathione (GSH) in the liver at **50 Fg/L** (24% reduction) and **340 Fg/L** (13% reduction). Consistent,

significant effects with rainbow trout were found at **50** and **340 Fg/L** (i.e., reductions of 15% and 14%, respectively, in protein levels in muscle); and at **350 Fg/L** (159% reduction in growth and a 23% increase in glucose levels) (MRID # 45202904).

Alazemi *et al*. (1996) reported gill damage to a freshwater fish; the damage was characterized by the presence of breaks in the gill epithelium at **500 Fg/L** which developed into deep pits at **5,000 Fg/L**.

Hussein *et al*. (1996) exposed two important Rile River fish (*Oreochromis niloticus* and *Chrysichthyes auratus*) to **3,000 and 6,000 Fg/L** atrazine for up to 28 days. Fish exposed to these concentrations showed some clinical signs such as rapid respiration and increased rate of gill cover movements; slower reflexes and swimming movements; reduction in feeding activities; loss of equibrium and death. These signs were more pronounced in *C. auratus* than *O. niloticus*. About 25 percent of the treated fish had abdominal swelling (ascites) in the two species. Abnormal behavior could be attributed to the effect of atrazine on CNS and cardiovascular system. Exposure to **3,000** and **6,000 Fg/L** resulted in significant ($p < 0.01$) decreases in the number of red blood cells (RBC), hemoglobin and haematocrit levels compared to controls in both species. While the data appear to show clear differences from controls, these conclusions could not be verified from the data given in the article . The authors also reported significant ($p < 0.01$) changes in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin (MCHC), serum components, and brain and serum AChE levels. While some of these measurements also appear to show clear differences between **3,000** and **6,000 Fg/L** and the controls, such as brain and serum AChE, whether the effects are significantly different than the controls could not be confirmed from the data presented in the article. (MRID # 45202911).

Neskovic *et al*. (1993) exposed carp to atrazine concentrations of 1,500, 3,000 and 6,000 Fg/L and and found biochemical changes in the activity of some enzyme activity levels in serum and some organs. Alkaline phosphatase levels were significantly ($p < 0.05$) higher in serum at all test levels than in controls. Alkaline phosphatase levels were lower, but not significantly ($p < 0.05$) less than control levels in the heart, liver and kidneys at all test levels. The greatest drop in alkaline phosphatase activity was found in the liver and ranged from 26.1% (**1,500 Fg/L**) to 50.2% (**6,000 Fg/L**). Somewhat weaker effects were found on glutamic-oxaloacetic (GOT) in the liver and kidney ($p < 0.1$). No statistically significant ($p < 0.01$) effects were found on glutamic-pyruvic transaminase (GPT). Histopathological effects include damage to gills ($\geq$ **1,500 Fg/L**), liver (almost normal at **1,500 Fg/L** and vacuolization of hepatocytes at $\geq$ **3,000 Fg/L**), kidney (more or less normal at **3,000 Fg/L** and with tubular epithelium and intertubular tissue degradation at **6,000 Fg/L**) and intestine (slightly greater lymphocyte infiltration and stronger mucous secretion at **6,000 Fg/L**) (MRID # 45202913).

iii. Freshwater Invertebrates, Acute

A freshwater aquatic invertebrate toxicity test using the TGAI is required to establish the toxicity of atrazine to aquatic invertebrates. The preferred test species is *Daphnia magna*. Results of this test and others are tabulated below.

Freshwater Invertebrate Acute Toxicity

Surrogate Species/ Static or Flow-through	% ai	96-hour LC50/EC50 Fg/L (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No.* Author/Year	Study Classification
Midge (*Chironomus tentans*) Static test	94	720 (nominal)	highly toxic	00024377 Macek *et al*. 1976	Supplemental (48-hour LC50 & raw data are missing)
Midge (*Chironomus riparius*)	85.5	1,000 (unknown)	highly toxic	45087413 Johnson 1986	Supplemental (raw data are missing)
Waterflea (*Daphnia magna*)	85.5	3,500 (unknown)	moderately toxic	45087413 Johnson 1986	Supplemental (raw data are missing)
Waterflea < 24-hours old (*Daphnia magna*) 26-Hour static test	??	3,600 (unknown)	at least moderately toxic	00002875 Frear & Boyd 1967	Supplemental (unknown ai, 26-hour test & no raw data)
Waterflea (*Ceriodaphnia dubia*) 48-Hour static test	97	> 4,900 (measured) Slope - no mortality	unknown	45208309 Jop 1991	Supplemental (EC50 value not determined)
Scud (*Gammarus fasciatus*) Static test	94	5,700 (nominal)	moderately toxic	00024377 Macek *et al*. 1976	Supplemental (48-hour LC50 & raw data are missing)
Stonefly (nymph) (*Acroneuria* sp.) Flow-through test 67.4 mg/L $CaCO_3$	98.5	6,700 (measured)	moderately toxic	??? Brooke 1990	Supplemental (study not seen; OW in draft WQC)
Waterflea (*Daphnia magna*) Static test	94	6,900 (nominal)	moderately toxic	00024377 Macek *et al*. 1976	Supplemental (raw data are missing)
Scud juvenile (*Hyalella azteca*) Flow-through test 67.4 mg/L Ca CO_3	98.5	14,700 (measured)	slightly toxic	??? Brooke 1990	Supplemental (no study; cited by OW in draft WQC)
Scud juvenile (*Gammarus pulex*) Static-renewal - daily	??	14,900 (measured) 4.4 @ 10 days	slightly toxic	45202917 Taylor, Maund & Pascoe 1991	Supplemental (raw data are missing)
Leech (*Glossiphonia complanata*) Static-renewal weekly	99.2	> 16,000 (measured) 6,300 Fg/L @ 28 days	slightly toxic	45202916 Streit & Peter 1978	Supplemental (raw data are missing)
Leech (*Helobdella stagnalis*) Static-renewal weekly	99.2	> 16,000 (measured) 9,900 Fg/L @ 27 days	slightly toxic	45202916 Streit & Peter 1978	Supplemental (raw data are missing)
Snail (*Ancylus fluviatilis*) Static-renewal weekly	99.2	>16,000 (measured) > 16, 000 Fg/L @ 40 days (35 % mortality)	slightly toxic	45208305 Oris, Winner & Moore 1991	Supplemental (raw data are missing)

Freshwater Invertebrate Acute Toxicity

Waterflea <12 hr old (*Ceriodaphnia dubia*) Static 48-hour test 57 mg/L $CaCO_3$	> 99	> 30,000 (measured) Slope - no data	slightly toxic	45202917 Taylor, Maund & Pascoe 1991	Supplemental (raw data are missing)
Midge (*Chironomus riparius*) Static-renewal - daily 10-Day test	??	> 33,000 (measured) 18,900 Fg/L @ 10 days	slightly toxic	00027204 Drake 1976	Supplemental (raw data are missing) (EC_{50} 115 ppm exceeds water solubility (33 ppm)
Formulations	% ai Product				
Waterflea (*Daphnia magna*) Flow-through test	79.6 80 WP	49,000 (higher concs. than 31,000 Fg/L were cloudy) (measured) slope 2.433	slightly toxic	42041401 Putt 1991	Supplemental for formulation (EC_{50} was not identified due to insolubility)
Waterflea (*Daphnia pulex*) Static test; 15EC 282 mg/L hardness With & without sediment	40.8 4 L	36,500 (nominal) 46,500 (with sediment)	slightly toxic	45227712 Hartman & Martin 1985	Supplemental for formulation (EC50 exceeds water solublity and low temp.)

* ??? indicates MRID no. pending

Since the lowest LC50/EC50 is in the range of 0.1 to 1 ppm, atrazine is categorized as highly toxic to aquatic invertebrates on an acute basis. The guideline requirement (72-2) is not fulfilled.

Degradates: The major atrazine degradate, hydroxyatrazine, forms a large percentage of the recoverable pesticide in aquatic compartments of the environment. Therefore, acute aquatic invertebrate testing with *Daphnia magna* is required to address degradate concerns. The requirement for the special degradate test (72-2) has not been fulfilled.

iv. Freshwater Invertebrate, Chronic

A freshwater aquatic invertebrate life-cycle test using the TGAI is required for atrazine since the end-use product is expected to be transported to water from the intended use site and the following conditions are met: the pesticide is intended for use such that its presence in water is likely to be continuous; an aquatic acute LC50 is less than 1 mg/L; and the pesticide is persistent in water (*i.e.*, half-life greater than 4 days). The preferred test species is *Daphnia magna*. Results of these tests are tabulated below.

Freshwater Aquatic Invertebrate Life-Cycle Toxicity

Surrogate Species/ Study Duration/ Flow-through or Static Renewal	% ai	NOEC/LOEC Fg/L (ppb) (measured or nominal)	Statistically sign. (p=0.05) Endpoints Affected	MRID No.* Author/Year	Study Classification

Scud (*Gammarus fasciatus*) 30 days / flow-through	94	NOAEC 60 LOAEC 140 (measured)	25 % red. in development of F_1 to seventh instar.	00024377 Macek *et al*. 1976	Core
Midge (*Chironomus tentans*) 38 days / flow-through	94	NOAEC 110 LOAEC 230 (measured)	25 % red. in F_0 pupation 29 % red. in F_0 adult emergence 18 % red. in F_1 pupation 28 % red. in F_1 adult emergence	00024377 Macek *et al*. 1976	Core
Waterflea (*Daphnia magna*) 21 days / flow-through	94	NOAEC 140 LOAEC 250 (measured)	54 % red. in F_0 young/female	00024377 Macek *et al*. 1976	Core
Waterflea (*Daphnia pulex*) 28-Day static-renewal	99.2	NOAEC 1,000 LOAEC 2,000 (nominal)	16 % sign. red. in young/adult	45202915 Schober & Lampert 1977	Supplemental (no raw data for statistical analyses)
70-Day static-renewal test			31 % red. in young/adult		
Waterflea - 6 generations (*Daphnia magna*) Static-renewal test	??	Cups: NOAEC 200 LOAEC 2,000 (unknown) 4 L aquarium: NOAEC ?? LOAEC ?? (water from treated corrals)	66 % reduction in # of young in generations 4, 5, & 6. 72% reduction in # of young	??? Kaushik, Solomon, Stephenson and Day 1985	Supplemental (methods and raw data are not reported)
Leech (*Helobdella stagnalis*) 40 Days Static-Renewal weekly	99.2	NOAEC <1,000 LOAEC 1,000 (measured)	65% red. in percent hatch	45202916 Streit & Peter 1978	Supplemental (no raw data for statistical analyses)
Waterflea < 12 hr. old (*Ceriodaphnia dubia*) Two 7-Day static-renewal tests; Renewed M, W, & F 57 $CaCO_3$; Temp. 25EC	> 99	NOAEC 2,500 LOAEC 5,000 NOAEC 2,500 LOAEC 5,000 (measured)	sign. red. in mean total number of young per living female (3 broods)	45208305 Oris, Winner and Moore 1991	Supplemental (no raw data for analyses)
Green hydra (normal) (*Chlorohydra viridissima*) 21-Day Static test	??	NOAEC <5,000 LOAEC 5,000 (nominal)	sign. red. in budding rates	45202901 Benson & Boush 1983	Supplemental (no raw data for analyses)
Waterflea 3-day old adult (*Ceriodaphnia dubia*) Two 4-Day static-renewal tests; Renewed M & W 57 $CaCO_3$; Temp. 25EC	> 99	NOAEC 5,000 LOAEC 10,000 NOAEC 10,000 LOAEC 20,000 (measured)	sign. red. in mean total number of young per living female (3 broods)	45208305 Oris, Winner and Moore 1991	Supplemental (no raw data for analyses)
Freshwater Snail (*Ancylus fluviatilis*) 40 Days Static-Renewal weekly	99.2	1,000 4,000 16,000 (measured)	38-39% red. in egg capsules & eggs in April/May 56-57% red. in eggs in April/May 15-16% red. in eggs in July/Aug. 68-73% red. in eggs in April/May 65-71% red. in eggs in July/Aug.	45202916 Streit & Peter 1978	Supplemental (no raw data for statistical analyses)
Leech (*Glossiphonia complanata*) 27-Days Static-Renewal weekly	99.2	1,000 4,000 16,000 (measured)	no reduction in egg production 17 % higher mortality 33 % higher mortality 67 % higher mortality	45202916 Streit & Peter 1978	Supplemental (no raw data for statistical analyses)

* ??? indicates MRID no. is pending

Growth stages and/or number of young are reduced by atrazine exposures for insects and crustaceans. The guideline requirement (72-4) is fulfilled (MRID 00024377). Streit and Peter (1978) concluded from the results from their study and literature data, that an exposure time of at least one month will exhibit significant toxic effects already at concentrations that are only 2 % of the acute 96 hour-LC_{50} value (MRID # 45202916).

Degradates: The major atrazine degradate is hydroxyatrazine which forms a large percent of the recoverable pesticide in aquatic compartments of the environment. Therefore, a special aquatic invertebrate life-cycle test (72-4) is reserved to address degradate concerns, pending the results of acute test.

v. Freshwater Field Studies

Kaushik *et al*. 1985 used large aquatic enclosures or limnocorrals to test the toxicity of atrazine on zooplankton and measure population recovery. The authors reported no significant numerical difference in the species level of cladoceran zooplankton between controls and atrazine-treated corrals. Dry weight estimates of individuals for 4 dominant zooplankton species (*Bosmina longirostris*, *Ceriodaphnia lacustris*, *Diaptomus oregonensis*, and *Mesocyclops edax*) from treated and untreated corrals did not differ significantly. The report lacks the raw data for statistical analyses. (MRID no. pending).

Walker (1964) treated Missouri ponds and plastic-lined limnocorrals with atrazine for aquatic weed control at levels of **500 to 2,000 Fg/L** and quantitatively examined effects on bottom organisms. Among the most sensitive organisms were mayflies (*Ephemeroptera*), caddis flies (*Tricoptera*), leeches (*Hirudinea*) and gastropods (*Musculium*). The most significant reduction in bottom fauna was observed during the period immediately following the application. Six to eight weeks after treatment, nine out of fourteen taxonomic groups had not recovered. While the total weight of the organisms per square foot was 31 percent higher than the controls, the total number of bottom organisms per square foot was 52 percent lower than in the controls. In addition, three categories (water bugs, mosquitoes, and leeches) were no longer present. (MRID # 45202919).

Streit and Peter (1978) reviewed Walker's findings and investigated long-term atrazine effects on three benthic freshwater invertebrates: *Ancylus fluviatilis* (Gastropoda - Basommatophora), *Glossiphonia complanata* and *Helobdella stagnalis* (both: Annelida - Hirudinea) in the laboratory (see Chronic Invertebrate toxicity table). Ingestion rates for *G. complanata* were determined over a 27-day period at atrazine concentrations of 1, 4 and 16 ppm. The total ingestion per individual was measured daily (except between Day 23 and 27). Two striking results were found: (1) Contaminated leeches ate significantly more limpets than the controls (300, 345 and 405% of control ingestion rates for **1,000**, **4,000** and **16,000 Fg/L** atrazine exposures, respectively). (2) All the lines are more or less straight, indicating that the ingestion rates were a constant intensity from immediately after the beginning of the exposure period. The same phenomenon was seen for snails, *A. fluviatilis*, but the intensity of feeding was much less (i.e., 120, 130 and 140% of control ingestion rates at **1,000**, **4,000** and **16,000 Fg/L,**

respectively). Other observations included: (1) leeches found sometimes lying on their backs suggesting that they have difficulty staying firmly attached to the substrate. (2) With increasing atrazine concentrations, an increasing percentage of snails could be detected that were just sucked out but not wholly eaten. Similar effects were observed with the snails which suggest that leech and snail behavior might be affected in some way. Compared to controls, *Ancylus* egg production was significantly reduced after 40 days exposure to atrazine at **16,000 Fg/L** in March/April, April/May (68% fewer egg capsules and 73% fewer eggs) and July/August (65% fewer egg capsules and 71% fewer eggs). Lower *Ancylus* reproduction was also found at **4 Fg/L** in April/May (56-57 percent) and July/August (15-16 percent). At **1,000 Fg/L**, fewer capsules and eggs were found only in April/May (38 and 39 percent, respectively). The average number of eggs per brood in leech, *Glossiphonia complanata* was not affected by 27-days of atrazine exposure. The no significant effect was found on the number of live-born young of *Helobdella stagnalis*. The eggs only developed completely normally in the control series. At **1,000** and **4,000 Fg/L** only a part of the egg masses developed. Only about 10 percent of the young in the **16,000 Fg/L** treatment hatched. Atrazine did not affect the time for normal development (5-6 days). Streit and Peter (1978) concluded from the results from their study and literature data that an exposure time of at least one month will exhibit significant toxic effects already at concentrations that are only 2 % of the acute 96 hour-LC_{50} value. (MRID # 45202916).

Kettle *et al*. (1987) monitored effects of atrazine (40.8%) on diet and reproductive success of bluegill in experimental, Kansas ponds. The 0.045-hectare, 2.1-meter deep ponds were each stocked with adult fish (50 bluegills, 20 channel catfish and 7 gizzard shad). On July 24, atrazine was applied to two ponds at **20 Fg/L**, another two ponds at **500 Fg/L** and two controls. Atrazine concentrations were measured during the study and 70% of the original concentration was detected at the end of the 136-day study. Bluegills were the only species to spawn during the study. Atrazine had no significant mortality on the original, stocked fish, but the number of young bluegills retrieved were significantly ($p \leq 0.01$) reduced compared to control ponds (i.e., 95.7 % fewer in **20 Fg/L**-treated ponds and 96.1 % fewer in **500 Fg/L**-treated ponds). Stomach analyses of adult bluegills indicate that the bluegill controls had significantly ($p \leq 0.001$) higher numbers of food items per fish stomach and higher numbers of prey taxa per fish stomach. The number of food items per stomach were reduced 85 and 78 percent in **20** and 500 Fg/L -treated ponds, respectively. Reductions in taxa per stomach were 57 and 52 percent in **20** and **500 Fg/L**-treated ponds, respectively. Stomachs of bluegills from treated ponds had fewer numbers of Ephemeroptera ($p \leq 0.001$), Odonata ($p \leq 0.001$), Coleoptera ($p \leq 0.01$) and Diptera (not significant, $p > 0.05$) than the controls. The macrophyte community in treated ponds was noticeably reduced, relative to controls, throughout the summer. Visual estimates of the macrophyte communities in the ponds showed roughly a 60 percent decline in the **20 Fg/L** ponds and a 90 percent decline in the **500 Fg/L** ponds two months after atrazine addition. These estimates were verified by rake hauls which produced these same relative differences. The following May, 10 months after treatment, when macrophytes are normally well established in Kansas ponds, the ponds were drained. Relative to control ponds, **20 Fg/L** ponds had a 90 percent reduction in macrophyte coverage and the **500 Fg/L** ponds had a >95 percent reduction in macrophyte coverage. Differences were noted in the macrophyte species present. Control ponds contained *Potamogeton pusillus* and *P. nodosus, Najas quadalupensis*, and small amounts

of *Chara globularis*, whereas the treated ponds contained mostly *C. globularis.* (MRID # 45202912).

c. Toxicity to Estuarine and Marine Animals

i. Estuarine and Marine Fish, Acute

Acute toxicity testing with estuarine/marine fish using the TGAI is required for atrazine because the end-use product is expected to reach this environment because of its use in coastal counties. The preferred test species is sheepshead minnow. Results of these tests are tabulated below.

Estuarine/Marine Fish Acute Toxicity

Surrogate Species/ Static or Flow-through/ Salinity & Temperature	% ai	96-hour LC50 (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification
Sheepshead Minnow larvae < 24-hours old (*Cyprinodon variegatus*) Static test, T - 20EC Salinity 25, 15, 5 g/L;	97.1	Sal. 25 g/L 2,000 Sal. 15 g/L 2,300 Sal. 5 g/L 16,200 (measured) Slope - no data	moderately toxic	45208303 & 45227711 Hall, Jr., Ziegenfuss, Anderson, Spittler & Leichtweis 1994	Supplemental (no raw data on mortalities)
Spot (*Leiostomus xanthurus*) Static test Salinity - 12 g/L; T - 22±1EC	97.4	8,500 (nominal) Slope - no data	moderately toxic	45202920 Ward & Ballantine 1985	Supplemental (no raw data)
Sheepshead minnow (*Cyprinodon variegatus*) Flow-through test Salinity - 31 g/L; T - 22-23EC	97.1	13,400 (measured) Slope 4.377	slightly toxic	43344901 Machado 1994	Core
Spot (juvenile) (*Leiostomus xanthurus*) Flow-through test Salinity - 29 g/L; T - 28EC	99.7	> 1,000 (nominal) Slope - none	unknown	40228401 Mayer 1986	Supplement (48-hour test)
Sheepshead minnow (*Cyprinodon variegatus*) Flow-through test	97.4	> 16,000 (30 % mortlity) (measured) Slope - none	unknown	45202920 Ward & Ballantine 1985	Supplemental (no raw data)

Since the LC50 are in the range of 1 - 10 ppm, Atrazine is categorized as moderately toxic to estuarine/marine fish on an acute basis. Toxicity data on sheepshead minnow, *Cyprinodon variegatus*, indicates that atrazine toxicity increases with increasing salinity levels. The pattern of increasing toxicity is opposite to atrazine toxicity data on the copepod, *Eurytemora affinis*. The guideline requirement (72-3a) is fulfilled (MRID 43344901).

Degradates: The major atrazine degradate, hydroxyatrazine, forms a large percentage of the recoverable pesticide in aquatic environmental compartments. Therefore, a special acute estuarine fish test (72-3) is required to address concerns for the toxicity of atrazine degradates to estuarine fish (preferably sheepshead minnow). The requirement (72-3) has not been fulfilled.

ii. Estuarine and Marine Fish, Chronic

An estuarine/marine fish early life-stage toxicity test using the TGAI is required for atrazine because the end-use product may be applied directly to the estuarine/marine environment or is expected to be transported to this environment from the intended use site, and the following conditions are met: the pesticide is intended for use such that its presence in water is likely to be continuous; an aquatic acute LC50 or EC50 is less than 1 mg/L; and the pesticide is persistent in water (*i.e.*, half-life greater than 4 days). The preferred test species is sheepshead minnow. Results of this test are tabulated below.

Estuarine/Marine Fish Early Life-Stage Toxicity Under Flow-through Conditions

Surrogate Species/ Study Duration/ Flow-through or Static Salinity & Temperature	% ai	NOAEC/LOAEC Fg/L (ppb) (measured or nominal)	Statistically sign. (p=0.05) Endpoints Affected	MRID No. Author/Year	Study Classification
Sheepshead Minnow (*Cyprinodon variegatus)* Study duration - unknown Flow-through test Salinity -13g/L; T 30±1EC	97.4	NOAEC 1,900 LOAEC 3,400 (measured)	89 % red. in juvenile survival	45202920 Ward & Ballantine 1985	Supplemental (no raw data for statistical analyses)

Biagianti-Risbourg and Bastide (1995) exposed juvenile gray mullets (*Liza ramada*) to 170 Fg/L atrazine for 9, 20, and 29 days in static tests and for 11 days followed by 18 days of decontamination; and then measured the sublethal effects on the liver. At **170 Fg/L**, 10, 25 and 60 percent mortality occurred following 9-, 20- and 29-day exposures, respectively; control mortality was a constant 10 percent throughout the test. Treated mullets showed normal behavior until Day 20 after which they stopped feeding and rapidly died; which is in contrast to the 90 percent survival of the treated fish that were transferred to clean water after 11 days of exposure. After 3-days exposure, a number of abnormalities were found in the liver (i.e., hepatic parenchyma with a few cytologically detectable perturbations; hepatocytes had particularly large lipofuscin granules; (MRID # 45204902). The guideline requirement (72-4) is not fulfilled.

Degradates: The major atrazine degradate, hydroxyatrazine, forms a large percentage of the recoverable pesticide in aquatic environmental compartments. Therefore, a special estuarine fish early life-stage test (72-4) is considered to address concerns for the toxicity of atrazine degradates to estuarine fish (preferably sheepshead minnow). The requirement (72-4) has not been fulfilled and is reserved pending the results of the acute estuarine fish test.

An estuarine/marine fish life-cycle test using the TGAI is reserved pending the results of acute and early life-stage tests on estuarine fish studies. The guideline requirements (72-5) is reserved.

iii. Estuarine and Marine Invertebrates, Acute

Acute toxicity testing with estuarine/marine invertebrates using the TGAI is required for atrazine because the end-use product is expected to reach this environment because of its use in coastal

counties. The preferred test species are mysid shrimp and eastern oyster. Results of these tests are tabulated below.

Estuarine/Marine Invertebrate Acute Toxicity

Surrogate Species/ Static or Flow-through/ Salinity & Temperature	% ai.	96-hour LC50/EC50 Fg/L (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification
Copepod (*Acartia tonsa*) Static-renewal - daily Salinity - 31 g/L; T 22EC	70 Tech.	88 (measured) Slope 0.947	very highly toxic	45202918 Thursby *et al.* 1990 memo	Supplemental (12% control mortality)
Copepod (*Acartia tonsa*) Static test Salinity - 20 g/L; T 20±1EC	97.4	94 (nominal) Slope - none	very highly toxic	45202920 Ward & Ballantine 1985	Supplemental (no raw data)
Copepod (*Acartia tonsa*) Static-renewal - daily Salinity - 31-32 g/L; T 22EC	70 Tech.	139 (measured) Slope 0.543	highly toxic	45202918 Thursby *et al.* 1990 memo	Supplemental (20% control mortality)
Copepod nauplii < 24 hours old (*Eurytemora affinis*) Static test; T - 20EC Salinity - 5, 15 & 25g/L	97.1	Sal. 5 g/L 500 Sal. 15 g/L 2,600 Sal. 25 g/L 13,300 (measured) Slope - no data	highly toxic to slightly toxic	45208303 & 45227711 Hall, Ziegenfuss, Anderson, Spittler & Leichtweis 1994	Supplemental (no raw data on mortality)
Mysid Shrimp (*Americamysis bahia*) Flow-through test Salinity 26 g/L; T 22±1EC	97.4	1,000 (Measured) Slope - none	highly toxic	45202920 Ward & Ballantine 1985	Supplemental (no raw data)
Brown Shrimp (juvenile) (*Penaeus aztecus*) Flow-through test Salinity - 30 g/L; T 27EC	99.7	1,000 (nominal) Slope - none	at least highly toxic	40228401 Mayer 1986	Supplemental (48-hr LC_{50} & no raw data)
Copepod - 17 days old (*Acartia tonsa*) Flow-through test Salinity - 31-33 /L, T - 20EC	97.1	4,300 (measured) Slope - 2.467	moderately toxic	45208308 McNamara 1991	Supplemental (cloudy with no 0.45 Fm filter of undissolved test material)
Mysid Shrimp (*Americamysis bahia*) Flow-through test Salinity -32 g/L; T 25-26EC	97.1	5,400 (measured) Slope 4.513	moderately toxic	43344902 Machado 1994	Core
Pink Shrimp (*Penaeus duorarum*) Static test Salinity 26 g/L; T 22±1EC	97.4	6,900 (nominal) Slope - none	moderately toxic	45202920 Ward & Ballantine 1985	Supplemental (no raw data)
Copepod (*Acartia clausii*) Static-renewal - daily Salinity - 31 g/L; T 6-6.2EC	70 Tech.	7,900 (nominal) Slope 0.958	moderately toxic	45202918 Thursby *et al.* 1990 memo	Core
Grass Shrimp (*Palaemonetes pugio*) Static test Salinity - 26 g/L; T 22±1EC	97.4	9,000 (nominal) Slope - none	Moderately toxic	45202920 Ward & Ballantine 1985	Supplemental (no raw data)

Estuarine/Marine Invertebrate Acute Toxicity

Surrogate Species/ Static or Flow-through/ Salinity & Temperature	% ai.	96-hour LC50/EC50 Fg/L (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification
Eastern oyster (juvenile) (*Crassostrea virginica*) (Shell deposition) Flow-through test Salinity - 28 g/L; T - 28EC	99.7	> 1,000 no effect (nominal) Slope - none	unknown	40228401 Mayer 1986	Supplemental (EC_{50} has not been identified & no raw data)
Mud Crab (*Neopanope texana*) Static test Salinity & T - unknown	Tech.	> 1,000 (nominal) Slope - none	slightly toxic	00024719 Bentley & Macek 1973	Supplemental (LC_{50} exceeds water solubility)

Since the lowest acute LC50/EC50 value is in the range of > 1 - 10 ppm, atrazine is categorized as moderately toxic to estuarine/marine invertebrates on an acute basis. Toxicity data on the copepod, *Eurytemora affinis*, indicates that atrazine toxicity decreases with increasing salinity levels. The pattern of decreasing toxicity is opposite to atrazine toxicity data on sheepshead minnows, *Cyprinodon variegatus*. The guideline requirement (72-3c) for shrimp is fulfilled (MRID 43344902), but the guideline requirement (72-3b) for oysters is not fulfilled.

Estuarine/Marine Invertebrate Acute Toxicity - Formulations

Surrogate Species/ Static or Flow-through	% ai. Product	96-hour LC50/EC50 Fg/L (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification
Eastern Oyster (*Crassostrea virginica*) (Shell deposition) Flow-through test Salinity -11.8 mg/L; T 21EC	79.6 80 WP	> 800 no effect (nominal) Slope - none	unknown	00024720 Wright & Beliles 1966	Supplemental (EC_{50} has not been identified)
Pacific Oyster (*Crassostrea gigas*) 24-Hour Static-Renewal	??	> 100 (nominal) 0.1 - 50% dead at 22 days 0.2 - 50% dead at 18 days	unknown	45227722 Moraga & Tanguy 2000	Supplemental (no 96-hour LC50 value)
European Brown Shrimp (*Crangon crangon*) Static test; 15EC	?? WP	10,000 - 33,000 (nominal) no slope	slightly toxic	45227728 Portmann 1972	Supplemental (only 48 hours & no raw data)
European Cockle (*Cardium edule*) Static test; 15EC	?? WP	> 100,000 (nominal) no slope	practically non-toxic	45227728 Portmann 1972	Supplemental (only 48 hours; LC_{50} exceeds water solubility & no raw data)
Fiddler Crab (*Uca pugilator*) Static test Salinity - 30 g/L; T 19EC	79.6 80 WP	198,000 (nominal) Slope - none	unknown	00024395 Union Carbide Corp. 1975	Supplemental (LC_{50} exceeds water solubility)

Estuarine/Marine Invertebrate Acute Toxicity - Formulations

Surrogate Species/ Static or Flow-through	% ai. Product	96-hour LC50/EC50 Fg/L (ppb) (measured/nominal) Probit Slope	Toxicity Category	MRID No. Author/Year	Study Classification
Fiddler Crab (*Uca pugilator*) Static test Salinity - 30 g/L; T 19EC	Unknown 4-1-3-1 WDL	239,000 (nominal) Slope - none	unknown	00024395 Union Carbide Corp. 1975	Supplemental (LC_{50} exceeds water solubility)

The toxicity of formulated atrazine products to marine/estuarine invertebrates are uncertain, because the EC/LC_{50} values are not definitive.

Degradates: The major atrazine degradate, hydroxyatrazine, forms a large percentage of the recoverable pesticide in aquatic environmental compartments. Therefore, estuarine invertebrate acute tests (72-3b and c) are required to address concerns for the toxicity of atrazine degradates to estuarine invertebrates (preferably *Americamysis bahia* and *Crassostrea virginica*). The requirement (72-3b and c) have not been fulfilled for any atrazine degradate.

iv. Estuarine and Marine Invertebrate, Chronic

An estuarine/marine invertebrate life-cycle toxicity test using the TGAI is required for atrazine because the end-use product may be applied directly to the estuarine/marine environment or is expected to be transported to this environment from the intended use site, and the following conditions are met: the pesticide is intended for use such that its presence in water is likely to be continuous and recurrent; an aquatic acute EC_{50} is less than 1 mg/L; and the pesticide is persistent in water (*e.g.*, half-life greater than 4 days). The preferred test species is mysid shrimp. Results of this test are tabulated below.

Estuarine/Marine Invertebrate Life-Cycle Toxicity

Species/ Duration/ Flow-through/ Static-renewal	% ai	NOAEC/LOAEC Fg/L (ppb) (measured/nominal)	Statistically sign. (P=0.05) Endpoints Affected	MRID No. Author/Year	Study Classification
Mysid *(Americamysis bahia)* Duration of test - unknown Flow-though test Salinity 20 g/L; 25+1EC	97.4	NOAEC 80 LOAEC 190 (measured)	37 % red. in adult survival	45202920 Ward & Ballantine 1985	Supplemental (no raw data for statistical analyses)

The guideline requirement (72-4b) for an estuarine invertebrate life-cycle test is not fulfilled.

Degradates: The major atrazine degradate, hydroxyatrazine, forms a large percentage of the recoverable pesticide in aquatic environmental compartments. Therefore, a special estuarine invertebrate life-cycle test (72-3) is required to address concerns for the toxicity of atrazine degradates to estuarine invertebrates (preferably *Americamysis bahia*). The requirement (72-4b)

has not been fulfilled for any atrazine degradate, but is reserved pending the results of the acute mysid test.

v. Estuarine and Marine Field Studies

Field studies are not available with atrazine effects on estuarine and/or marine animals. No estuarine field studies are required.

d. Toxicity to Plants

I. Terrestrial

Terrestrial plant testing (seedling emergence and vegetative vigor) is required for all herbicides.

For seedling emergence and vegetative vigor testing the following plant species and groups should be tested: (1) six species of at least four dicotyledonous families, one species of which is soybean (*Glycine max*) and the second is a root crop, and (2) four species of at least two monocotyledonous families, one of which is corn (*Zea mays*).

Results of toxicity testing on the technical material are tabulated below.

Nontarget Terrestrial Plant Seedling Germination Toxicity (Tier II)

Surrogate Species	% ai	EC25/EC05 (lbs ai/A) Probit Slope	Endpoint Affected	MRID No. Author/Year	Study Classification
Monocot - Corn (*Zea mays*)	97.7	< 4.0 / < 4.0	No effect	41223001 Chetram 1989	Core
Monocot - Oat (*Avena sativa*)	97.7	1.8 / 0.12 slope 0.834	% red..in radicle length	41223001 Chetram 1989	Core
Monocot - Onion (*Allium cepa*)	97.7	< 4.0 /< 4.0	No effect	41223001 Chetram 1989	Core
Monocot - Ryegrass (*Lolium perenne*)	97.7	< 4.0 / < 4.0 slope 0.834	No effect	41223001 Chetram 1989	Core
Dicot - Root Crop - Carrot (*Daucus carota*)	97.7	<4.0 / < 4.0	No effect	41223001 Chetram 1989	Core
Dicot - Soybean (*Glycine max*)	97.7	< 4.0 / < 4.0	No effect	41223001 Chetram 1989	Core
Dicot - Lettuce (*Lactuca sativa*)	97.7	< 4.0 / < 4.0	No effect	41223001 Chetram 1989	Core
Dicot - Cabbage (*Brassica oleracea alba*)	97.7	< 4.0 / < 4.0	No effect	41223001 Chetram 1989	Core
Dicot - Tomato (*Lycopersicon esculentum*)	97.7	< 4.0 / < 4.0	No effect	41223001 Chetram 1989	Core
Dicot - Cucumber (*Cucumis sativus*)	97.7	0.80 / 0.60 slope 0.864	% red. in radicle length	41223001 Chetram 1989	Core

Results from the Tier II seedling germination tests indicate that cucumber is the most sensitive dicot and oats is the most sensitive monocot. These studies are acceptable (MRID 41223001), but the guideline requirement for seed germination testing has now been included in the seedling emergence toxicity test.

Nontarget Terrestrial Plant Seedling Emergence Toxicity (Tier II)

Surrogate Species	% ai	EC25 / NOAEC (lbs ai/A)	Endpoint Affected	MRID No. Author/Year	Study Classification
Monocot - Corn (*Zea mays*)	97.7	> 4.0 / > 4.0	No effect	42041403 Chetram 1989	Core
Monocot - Oat (*Avena sativa*)	97.7	0.004 / 0.0025	red. in dry weight	42041403 Chetram 1989	Core
Monocot - Onion (*Allium cepa*)	97.7	0.009 / 0.005	red. in dry weight	42041403 Chetram 1989	Core
Monocot - Ryegrass (*Lolium perenne*)	97.7	0.004 / 0.005	red. in dry weight	42041403 Chetram 1989	Core
Dicot - Root Crop - Carrot (*Daucus carota*)	97.7	0.003 / 0.0025	red. in dry weight	42041403 Chetram 1989	Core
Dicot - Soybean (*Glycine max*)	97.7	0.19 / 0.025	red. in dry weight	42041403 Chetram 1989	Core
Dicot - Lettuce (*Lactuca sativa*)	97.7	0.005 / 0.005	red. in dry weight	42041403 Chetram 1989	Core
Dicot - Cabbage (*Brassica oleracea alba*)	97.7	0.014 / 0.01	red. in dry weight	42041403 Chetram 1989	Core
Dicot - Tomato (*Lycopersicon esculentum*)	97.7	0.034 / 0.01	red. in dry weight	42041403 Chetram 1989	Core
Dicot - Cucumber (*Cucumis sativus*)	97.7	0.013 / 0.005	red. in dry weight	42041403 Chetram 1989	Core

For Tier II seedling emergence, the most sensitive dicot is the carrot and the most sensitive monocots are oat and ryegrass. The guideline requirement (123-1a) is fulfilled (MRID 42041403).

Nontarget Terrestrial Plant Vegetative Vigor Toxicity (Tier II)

Surrogate Species	% ai	EC25 / NOAEC (lbs ai/A)	Endpoint Affected	MRID No. Author/Year	Study Classification
Monocot - Corn (*Zea mays*)	97.7	< 4.0 / < 4.0	No effect	42041402 Chetram 1989	Core
Monocot - Oat (*Avena sativa*)	97.7	2.4 / 2.0	red. in dry weight	42041402 Chetram 1989	Core
Monocot - Onion (*Allium cepa*)	97.7	0.61 / 0.5	red. in dry weight	42041402 Chetram 1989	Core
Monocot - Ryegrass (*Lolium perenne*)	97.7	< 4.0 / < 4.0	No effect	42041402 Chetram 1989	Core
Dicot - Root Crop - Carrot (*Daucus carota*)	97.7	1.7 / 2.0	red. in plant height	42041402 Chetram 1989	Core

Nontarget Terrestrial Plant Vegetative Vigor Toxicity (Tier II)

Surrogate Species	% ai	EC25 / NOAEC (lbs ai/A)	Endpoint Affected	MRID No. Author/Year	Study Classification
Dicot - Soybean (*Glycine max*)	97.7	0.026 / 0.02	red. in dry weight	42041402 Chetram 1989	Core
Dicot - Lettuce (*Lactuca sativa*)	97.7	0.33 / 0.25	red. in dry weight	42041402 Chetram 1989	Core
Dicot - Cabbage (*Brassica oleracea alba*)	97.7	0.014 / 0.005	red. in dry weight	42041402 Chetram 1989	Core
Dicot - Tomato (*Lycopersicon esculentum*)	97.7	0.72 / 0.5	red. in plant height	42041402 Chetram 1989	Core
Dicot - Cucumber (*Cucumis sativus*)	97.7	0.008 / 0.005	red. in dry weight	42041402 Chetram 1989	Core

For Tier II vegetative vigor, the most sensitive dicot is cucumber and the most sensitive monocot is onion. The guideline requirement (123-1b) is fulfilled (MRID 42041402).

ii. Aquatic Plants

Aquatic plant testing is required for all herbicides. The following species should be tested at Tier II: *Kirchneria subcapitata* (formally *Selenastrum capricornutum*, *Lemna gibba*, *Skeletonema costatum*, *Anabaena flos-aquae*, and a freshwater diatom. Aquatic plant testing is required for atrazine because atrazine is applied on crops outdoors and would appear to be mobile with a water solubility value of 33 ppm.

Results of Tier II toxicity testing on technical grade and typical end-use products (TEP) are tabulated below. The data are presented in four toxicity tables separating the freshwater data from the marine data and the short, 7-day or less tests from the longer tests.

Nontarget Freshwater Plant Toxicity (Tier II)

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No.* Author/Year	Study Classification
Vascular Plants:					
Duckweed (*Lemna gibba*) 5-Day test; Static-Renewal	97	170 (nominal) Slope 3.93	50% red. in growth	41065203d Hughes 1986	Supplemental (5 days, not 14 days)
Duckweed (*Lemna gibba*) 7-Day test; Static-Renewal	97	170 (measured) Slope 2.2	50% red. in growth	42041404 Hoberg 1991	Core
Non-Vascular Plants:					
Cyanophyceae *Oscillatoria lutea* (1week; nominal)	76 80 W	< 1 1,000	93% red. chlorophyll production 100% red. chlorophyll production	00023544 Torres and O'Flaherty 1976	Supplemental (raw data unavailable)

Nontarget Freshwater Plant Toxicity (Tier II)

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No.* Author/Year	Study Classification
Chlorophyceae *Stigeoclonium tenue* (1 week; nominal)	76 80 W	< 1 1,000	67% red. chlorophyll production 90% red. chlorophyll production	00023544 Torres and O'Flaherty 1976	Supplemental (raw data unavailable)
Green Algae - Chlorophyceae *Chlorella vulgaris* (1 week; nominal)	76 80 W	1 1,000	50% red. chlorophyll production 80-87% red. chlorophyll production	00023544 Torres and O'Flaherty 1976	Supplemental (raw data unavailable)
Xanthophyceae *Vaucheria geminata* (1 week; nominal)	76 80 W	1 1,000	41% red. chlorophyll production 100% red. chlorophyll production	00023544 Torres and O'Flaherty 1976	Supplemental (raw data unavailable)
Chlorophyceae *Chlamydomonas reinhardi* (24 hour; nominal)	Unk.	19 44 48	50% red. carbon uptake; media: Taub & Dollar (TD)	45020015 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Chlorophyceae *Kirchneria subcapitata* =*Selenastrum capricornutum* (96 hours; nominal)	Tech.	26 26	50% red. cell growth 50% red. floresence	??? Caux, Menard, and Kent 1996	Supplemental (NOAEC and raw data unavailable)
Chlorophyceae *Kirchneria subcapitata* = *Selenastrum capricornutum* (24 hours; nominal)	Unk.	34 42 53	50% red. 14-carbon uptake; media: Taub & Dollar (TD); algal assay & TD, respect.	45020015 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Cyanophyceae *Anabaena cylindrica* (?? hours; nominal)	97	37	50% red. in photosynthesis	??? Stratton & Corke 1981	Supplemental (no raw data)
Chlorophyceae *Scenedesmus obliquus* (24 hour; nominal)	Unk.	38 49 57	50% red. 14-carbon uptake; media: Taub & Dollar (TD)	45020015 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Chlorophyceae *Kirchneria subcapitata* =*Selenastrum capricornutum* (120 hours; measured)	97.1	49 NOAEC 16 Slope 4.002	50% red. cell growth	43074802 Hoberg 1993	Core
Cyanophyceae *Anabaena inaequalis* (?? hours; nominal)	97	50	50% red. in photosynthesis	??? Stratton & Corke 181	Supplemental (no raw data)
Chlorophyceae *Kirchneria subcapitata* = *Selenastrum capricornutum* (120 hours; nominal)	97.4	53 NOAEC <32 LOAEC 32 Slope 4.127	50% red. growth 17% red. growth	41065204 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)
Bacillariophyceae Navicula pelliculosa (120 hours; nominal)	97.1	60 NOAEC <10 LOAEC 10 Slope 2.31	50% red. growth	41065203a Hughes 1986	Core (EC50 extrapolated; and NOAEC was not determined)
Chlorophyceae *Ankistrodesmus* sp. (24 hours; nominal)	Unk.	61 72 219	50% red. 14-carbon uptake; media: Taub & Dollar (TD), TD & algal assay, respect.	45020015 Larsen *et al*. 1986	Supplemental (raw data unavailable)

Nontarget Freshwater Plant Toxicity (Tier II)

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No.* Author/Year	Study Classification
Ulothrix subconstricta Tentative species identification (24 hours; nominal)	Unk.	88	50% red. 14-carbon uptake; medium: Taub & Dollar (TD)	45020015 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Cyanophyceae *Anabaena variabilis* (?? hours; Nominal)	97	100	50% red. in photosnythesis	??? Stratton & Corke 1981	Supplemental (no raw data)
Stigeoclonium tenue Tentative species Identification (24 hours; nominal)	Unk.	127 224	50% red. 14-carbon uptake; media: Taub & Dollar (TD)	45020015 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Chlorophyceae *Kirchneria subcapitata* =*Selenastrum capricornutum* (96 hours; measured)	97	130 NOAEC 76 Slope 6.628	50% red. cell growth	42060701 Hoberg 1991	Supplemental (higher light intensity than recommended)
Cyanophyceae *Anabaena cylindrica* (24 hour; nominal)	Unk.	178 182 253	50% red. 14-carbon uptake; media: Taub & Dollar (TD), algal assay, & TD, respect.	45020015 Larsen *et al*. 1986	Supplemental (raw data unavailable)
Cyanophyceae *Anabaena flos-aquae* (120 hours; nominal)	97	230 NOAEC <100 LOAEC 100 Slope 1.95	50% red. growth 22% red. growth	41065203a Hughes 1986	Core (NOAEC was not determined)
Chlorophyceae *Chlorella pyrenoidosa* (120 hours; nominal)	97.4	282 NOAEC 130 Slope 4.216	50% red. growth 7% red. growth	41065204 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)
Chlorophyceae *Chlorella vulgaris* (24 hours; nominal)	Unk.	293 305 325	50% red. 14-carbon uptake; media: Algal assay, Taub & Dollar (TD), & TD, respect.	45020015 Larsen *et al*. 1986	Supplemental (raw data unavailable)

Longer Term, Nontarget Freshwater Plant Toxicity

Surrogate Species/ Duration/ Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No.* Author/Year	Study Classification
Vascular Plants:					
Broad Waterweed *Elodea canadensis* (20 days; measured)	????	NOAEC 2 LOAEC 10	200% incr. dark respiration 33% incr. net photosynthesis	??? Hofmann and Winkler 1990	Supplemental (raw data unavailable)
Pondweed *Potamogeton perfoliatus* (4 weeks; initial conc. nominal, terminal conc. measured)	???	30 Week 3: LOAEC 5 NOAEC < 5 4 Weeks: LOAEC 50 NOAEC 5	50% red. O_2 product. sign. red. O_2 product. sign. red. O_2 product.	??? Kemp *et al*. 1985	Supplemental (raw data unavailable)

Longer Term, Nontarget Freshwater Plant Toxicity

Surrogate Species/ Duration/ Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No.* Author/Year	Study Classification
Duckweed *Lemna gibba* (14 days; measured)	97.1	37 LOAEC 3.4 NOAEC < 3.4 Slope 1.716	50% red. growth 19% red. growth (frond production)	43074804 Hoberg 1993	Supplemental (NOAEC was not determined)
Duckweed - *Lemna gibba* (14 days; measured)	97.4	43 NOAEC 10 Slope 1.995	50% red. growth (frond production)	43074803 Hoberg 1993	Core
Broad Waterweed *Elodea canadensis* (3 weeks; nominal)	???	80	50% red. shoot length	45087410 Forney and Davis 1981	Supplemental (raw data unavailable)
Eurasian Water-Milfoil *Myriophyllum spicatum* (4 weeks; initial conc. nominal, terminal conc. measured)	????	91 NOAEC 5 LOAEC 50	50% red, O_2 product. Sign. red. O_2 product.	??? Kemp *et al*. 1985	Supplemental (raw data unavailable)
Non-Vascular Plants:					
36 freshwater algal strains (2 weeks; nominal)	99.0	10 1,000	growth <than control strong growth red.	??? Butler *et al*. 1975	Supplemental (raw data unavailable)
Chlorophyceae *Chlorella vulgaris* (11 days; nominal)	99.9	25	50% red. cell growth	45227703 Burrell *et al. 1985*	Supplemental (raw data unavailable)
Cyanophyceae *Anabaena inaequalis* (12-14 days[1]; nominal)	>95	30 100 300	50% red. cell count 50% red. growth rate 50% red. photosynthesis	45087401 Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Chlorophyceae *Ankistrodesmus braunii* (11 days; nominal)	99.9	60	50% red. cell growth	45227703 Burrell *et al*. 1985	Supplemental (raw data unavailable)
Chlorophyceae *Scenedesmus quadricauda* (12-14 days[1]; nominal)	> 95	100 200 300	50% red. cell count 50% red. growth rate 50% red. photosynthesis	45087401 Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Chlorophyceae *Chlorella pyrenoidosa* (12-14 days[1]; nominal)	> 95	300 1,000 500	50% red. cell count 50% red. growth rate 50% red. photosynthesis	??? Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Cyanophyceae *Anabaena cylindrica* (12-14 days; nominal)	> 95	1,200 3,600 500	50% red. cell count 50% red. growth rate 50% red. photosynthesis	??? Stratton 1984	Supplemental (NOAEC and raw data unavailable)
Cyanophyceae *Anabaena variabilis* (12-14 days; nominal)	> 95	4,000 5,000 100	50% red. cell count 50% red. growth rate 50% red. photosynthesis	??? Stratton 1984	Supplemental (NOAEC and raw data unavailable)

* ??? indicates MRID no. is pending

Nontarget Marine/Estuarine Plant Toxicity (Tier II)

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Vascular Plants:					
Fontinalis sp. (24 hours; measured)	????	NOAEC 2 LOAEC 10	red. net O_2 production		Supplemental (raw data unavailable)
Pondweed (Estuarine) *Potamogeton perfoliatus* (2 hours; nominal)	????	77	50% red. O_2 evolution	45227718 Jones and Winchell 1984	Supplemental (Insufficient duration; raw data unavailable)
Pondweed *Potamogeton perfoliatus* (2 hours; nominal)	???	80 650	50% red. O_2 product. 87% red. O_2 product..	45227718 Jones *et al*. 1986	Supplemental (Insufficient duration; raw data unavailable)
Zannichellia palustris (2 hours; nominal)	????	91	50% red. O_2 evolution	45227719 Jones and Winchell 1984	Supplemental (Insufficient duration; raw data unavailable)
Pondweed (Estuarine) *Potamogeton perfoliatus* (2 hours; nominal)	Unk.	100	52 to 69% red. in photosynthesis	45087404 Jones & Estes 1984	Supplemental (raw data unavailable)
Widgeon-Grass (Estuarine) Ruppia maritima (2 hours; nominal	?????	102	50% red. O_2 evolution	45227719 Jones and Winchell 1984	Supplemental (Insufficient duration; raw data unavailable)
Non-Vascular Plants:					
Chrysophyceae *Isochrysis galbana* (120 hours; nominal)	97.4	22 NOAEC < 13 LOAEC 13 Slope 3.065	50% red. growth 30% red. growth	41065204 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)
Marine Diatom *Skeletonema costatum* (120 hours; nominal)	97.4	24 NOAEC < 13 LOAEC 13 Slope 3.343	50% red. growth 14% red. growth	41065204 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)
Marine Diatom Skeletonema costatum (120 hours; measured)	97.1	53 NOAEC 14 Slope 2.798	50% red. cell growth	43074801 Hoberg 1993	Core
Marine Green - Chlorophyceae *Chlamydomonas* sp. (72 hours; nominal); Salinity 30 g/L	99.7	60	50% red. O_2 production	40228401 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Yellow - Chrysophyceae *Monochrysis lutheri* (72 hours; nominal); Salinity 30 g/L	99.7	77	50% red. O_2 production	40228401 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Red - Rhodophyceae *Porphyridium cruentum* (72 hours; nominal); Salinity 30 g/L	99.7	79	50% red. in O_2 production	40228401 Mayer 1986	Supplemental (72 hrs & endpoint)

Nontarget Marine/Estuarine Plant Toxicity (Tier II)

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Marine Green - Chlorophyceae *Neochloris* sp. (72 hours; nominal); Salinity 30 g/L	99.7	82	50% red. in O_2 production	40228401 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Bacillariophyceae Cyclotella nana (72 hours; nominal); Salinity 30 g/L	99.7	84	50% red. in O_2 production	40228401 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Bacillariophyceae *Achnanthes brevipes* (72 hours; nominal); Salinity 30 g/L	99.7	93	50% red. in O_2 production	40228401 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Yellow - Chrysophyceae *Isochrysis galbana* (240 hours; nominal); Salinity 30 g/L	99.7	100	50% red. cell growth	40228401 Mayer 1986	Supplemental (NOAEC unavailable)
Marine Green - Chlorophyceae *Chlorococcum* sp. (240 hours; nominal); Salinity 30 g/L	99.7	100	50% red. cell growth	40228401 Mayer 1986	Supplemental (NOAEC unavailable)
Marine Green - Chlorophyceae *Platymonas* sp. (72 hours; nominal); Salinity 30 g/L	99.7	100	50% red. O_2 production	40228401 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Bacillariophyceae *Thalassiosira fluviatilis* (72 hours; nominal); Salinity 30 g/L	99.7	110	50% red. O_2 production	40228401 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Bacillariophyceae *Stauroneis amphoroides* (72 hours; nominal); Salinity 30 g/L	99.7	110	50% red. O_2 production	40228401 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Algae *Microcystis aeruginosa* (120 hours - nominal)	97.4	129 NOAEC 65 Slope 3.162	50% red. growth 7% red. growth	41065204 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)
Marine Green - Chlorophyceae *Chlorella* sp. (72 hours; nominal); Salinity 30 g/L	99.7	140	50% red. O_2 production	40228401 Mayer 1986	Supplemental (NOAEC unavailable
Blue-green - Cyanophyceae *Anabaena cylindrica* (24 hour; nominal)	Unk.	178 182 253	50% red. 14-carbon uptake; media: Taub & Dollar (TD), algal assay, & TD, respect.	45020015 Larsen *et al*. 1986	Supplemental (raw data unavailable)

Nontarget Marine/Estuarine Plant Toxicity (Tier II)

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Marine green - Chlorophyceae *Dunaliella tertiolecta* (120 hours; nominal)	97	180 NOAEC < 100 LOAEC 100 Slope 1.95	50% red. growth 34% red. growth	41065203 Hughes 1986	Supplemental (NOAEC unavailable)
Marine Yellow - Chrysophyceae Phaeodactylum tricornutum (240 hours; nominal); Salinity 30 g/L	99.7	200	50% red. cell growth	40228401 Mayer 1986	Supplemental (NOAEC unavailable)
Marine Bacillariophyceae *Nitzschia closterium* (72 hours; nominal); Salinity 30 g/L	99.7	290	50% red. O_2 production	40228401 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Bacillariophyceae *Amphora exigua* (72 hours; nominal); Salinity 30 g/L	99.7	300	50% red. O_2 production	40228401 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Green - Chlorophyceae *Dunaliella tertiolecta* (240 hours; nominal); Salinity 30 g/L	99.7	300	50% red. cell growth	40228401 Mayer 1986	Supplemental (NOAEC unavailable)
Marine Red - Rhodophyceae *Porphyridium cruentum* (120 hours)	97.4	308 NOAEC <130 LOAEC 130 Slope 2.449	50% red. growth 16% red. growth	41065204 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)
Marine Bacillariophyceae *Nitzschia* (Ind. 684) (72 hours; nominal); Salinity 30 g/L	99.7	430	50% red. O_2 production	40228401 Mayer 1986	Supplemental (72 hrs & endpoint)
Marine Green -Chlorophyceae *Kirchneria subcapitata* (120 hours; nominal)	97.4	431 NOAEC 200 Slope 4.217	5% red. in growth 4% red. in growth	41065204 Parrish 1978	Supplemental (NOAEC, method & raw data unavailable)
Marine Bacillariophyceae *Navicula inserta* (72 hours; nominal); Salinity 30 g/L	99.7	460	50% red. in O_2 production	40228401 Mayer 1986	Supplemental (72 hrs & endpoint)

Formulation Nontarget Marine/Estuarine Algal Toxicity (Tier II)

Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit slope	% Response	MRID No. Author/Year	Study Classification
Mar. Yellow - Chrysophyceae *Isochrysis galbana* (nominal); Salinity 30 g/L	76 80 WP	100 (240 hrs) 200 (2 hrs)	50% red. cell growth 50% red. O_2 production	40228401 Mayer 1986	Supplemental (NOAEC unavailable)

Formulation Nontarget Marine/Estuarine Algal Toxicity (Tier II)

Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit slope	% Response	MRID No. Author/Year	Study Classification
Mar. Yellow Chlorophyceae *Chlorococcum* sp. (nominal); Salinity 30 g/L	76 80 WP	100 (240 hrs) 400 (2 hrs)	50% red. cell growth 50% red. O_2 production	40228401 Mayer 1986	Supplemental (NOAEC unavailable)
Mar. Yellow - Chrysophyceae Phaeodactylum tricornutum (nominal); Salinity 30 g/L	76 80 WP	200 (240 hrs) 200 (2 hrs)	50% red. cell growth 50% red. O_2 production	40228401 Mayer 1986	Supplemental (NOAEC unavailable)
Mar. Green - Chlorophyceae *Dunaliella tertiolecta* (nominal); Salinity 30 g/L	76 80 WP	400 (240 hrs) 600 (2 hrs)	50% red. cell growth 50% red. O_2 production	40228401 Mayer 1986	Supplemental (NOAEC unavailable)

Longer-term Nontarget Marine/Estuarine Plant Toxicity

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Vascular Plants:					
Sago Pondweed (Estuarine) *Potamogeton pectinatus* (28 days; measured/nominal)	???	Salinity 12 ppt: NOAEC 7.5 LOAEC 14.3 Salinity 1 & 6 ppt: NOAEC 14.3 LOAEC 30	 sign. red. dry weight sign. red. dry weight	45088231 Chesapeake Bay Program 1998	Supplemental (raw data unavailable)
Estuarine rush *Juncus roemerianus* (5 weeks - 1 year; measured	97.1	LOAEC 30 NOAEC 30 NOAEC < 30 250 ppb 3, 800 ppb	sign. red. chlorophyll a in 5 weeks (1 year) partial recovery (1 yr) practically no survival	45087405 Lytle & Lytle 1998	Supplemental (raw data unavailable)
Pondweed *Potamogeton perfoliatus* (4 weeks; initial conc. nominal, terminal conc. measured)	???	30 Week 3: LOAEC 5 NOAEC < 5 4 weeks: LOAEC 50 NOAEC 5	50% red. O_2 product. sign. red. O_2 product. sign. red. O_2 product.	45227720 Kemp *et al*. 1985	Supplemental (raw data unavailable)
Pondweed (Estuarine) *Potamogeton perfoliatus* (3 weeks; nominal)	???	53	50% red. ????	45087410 Forney and Davis 1981	Supplemental (raw data unavailable)
Eelgrass (Estuarine) *Zostera marina* (10 days; measured)	Unk.	est. 69 50 80	50% red. leaf growth 25% red. leaf growth 62% red. leaf growth	45227729 Schwarzschild *et al*. 1994	Supplemental (raw data unavailable)
Estuarine Eelgrass *Zostera marina* (21 days; nominal)	???	100 NOAEC 10	21-day LC50 red. production	45227705 Delistraty and Hershner 1984	Supplemental (raw data unavailable)
Wild Celery (Estuarine) *Vallisneria americana* (6 weeks; nominal)	???	163	50% red. shoot length no difference at 0, 3, or 6 parts/thousand	45087410 Forney and Davis 1981	Supplemental (raw data unavailable)

Longer-term Nontarget Marine/Estuarine Plant Toxicity

Surrogate Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit Slope	% Response	MRID No. Author/Year	Study Classification
Seagrass (Estuarine) *Halodule wrightii* (22 - 23 days; measured)	Atrazine 4L	30,000	46-58% red. total above-ground biomass	45205101 Mitchell 1987	Supplemental (raw data unavailable)
Non-Vascular Plants:					
Marine Brown macroalgae *Laminaria hyperborea* (18 days; nominal)	???	NOAEC < 10 LOAEC 10 50 & 100	sign. red. growth rate delayed sporophyte formation	??? Hopkin &Kain 1978	Supplemental (raw data unavailable)
Marine Yellow - Chrysophyceae *Isochrysis galbana* (240 hours; nominal); Salinity 30 g/L	99.7	100	50% red. cell growth	40228401 Mayer 1986	Supplemental (NOAEC unavailable)
Marine Green - Chlorophyceae *Chlorococcum* sp. (240 hours; nominal); Salinity 30 g/L	99.7	100	50% red. cell growth	40228401 Mayer 1986	Supplemental (NOAEC unavailable)
Marine Yellow - Chrysophyceae Phaeodactylum tricornutum (240 hours; nominal); Salinity 30 g/L	99.7	200	50% red. cell growth	40228401 Mayer 1986	Supplemental (NOAEC unavailable)
Marine Green - Chlorophyceae *Dunaliella tertiolecta* (240 hours; nominal); Salinity 30 g/L	99.7	300	50% red. cell growth	40228401 Mayer 1986	Supplemental (NOAEC unavailable)

* ??? indicates MRID no. pending

The Tier II results indicate that the marine algae *Isochrysis galbana* is the most sensitive nonvascular aquatic plant (EC_{50} 22 ppb) and the most sensitive vascular aquatic plant is wild celery (4 ppb). Toxicity of the atrazine formulation 80 WP was usually the same as the cell growth values for the technical grade. Comparison of atrazine toxicity levels for three different endpoints suggest that the endpoints in decreasing order of sensitivity are cell count, growth rate and oxygen production (Stratton 1984). Walsh (1983) exposed *Skeletonema costatum* to atrazine and concluded that atrazine is only slightly algicidal at relatively high concentrations (i.e., 500 & 1,000 ppb). Caux *et al*. (1996) compared the cell count IC_{50} and fluorescence LC_{50} and concluded that atrazine is algicidal at concentrations which effect cell counts. Abou-Waly *et al*. (1991) measured growth rates on days 3, 5, and 7 for two algal species. The pattern of atrazine effects on growth rates differ sharply between the two species. Atrazine had a strong early effect on *Anabaena flos-aquae* followed by rapid recovery in clean water (i.e., EC_{50} values for days 3, 5, and 7 are 58, 469, and 766 ppb, respectively). The EC_{50} values for *Selenastrum capricornutum* continued to decline from Day 3 through 7 (i.e., 283, 218, and 214 ppb, respectively. Based on theses results, it appears that the timing of peak effects for atrazine may

differ depending on the test species. The guideline requirement (123-2) is fulfilled for only three out of five species (MRID 43074801, 43074802, 43074803). However, sufficient data exists on numerous other algal species to provide a broad range of toxicity effects. No additional algal studies are required.

Degradates: The major atrazine degradate is hydroxyatrazine which forms a large percent of the recoverable pesticide in aquatic compartments of the environment. Therefore, special tests are required for algal and vascular plant species (123-2) to address concerns for the toxicity of atrazine degradates to aquatic plants.

Degradate Deethylatrazine Nontarget Aquatic Plant Toxicity (Tier II)

Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit slope	% Response	MRID No.* Author/Year	Study Classification
Fresh. Blue-Green - Cyanophyceae	> 95	1,000	50% red. cell count	???	Supplemental
Anabaena inaequalis		4,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days[1]; nominal)		2,500	50% red. photosynthesis		unavailable)
Freshwater Green - Chlorophyceae	> 95	1,200	50% red. cell count	???	Supplemental
Scenedesmus quadricauda		2,000	50% red. Growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		1,800	50% red. photosynthesis		unavailable)
Freshwater Green - Chlorophyceae	> 95	3,200	50% red. cell count	???	Supplemental
Chlorella pyrenoidosa		7,200	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days[1]; nominal)		1,800	50% red. photosynthesis		unavailable)
Fresh. Blue-Green - Cyanophyceae	> 95	3,500	50% red. cell count	???	Supplemental
Anabaena variabilis		7,500	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		700	50 % red. photosynthesis		unavailable)
Fresh. Blue-Green - Cyanophyceae	> 95	8,500	50% red. cell count	???	Supplemental
Anabaena cylindrica		5.500	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		4,800	50% red. photosynthesis		unavailable)

Degradate Deisopropylatrazine Nontarget Aquatic Plant Toxicity (Tier II)

Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit slope	% Response	MRID No. Author/Year	Study Classification
Fresh. Blue-Green - Cyanophyceae	> 95	2,500	50% red. cell count	???	Supplemental
Anabaena inaequalis		7,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days[1]; nominal)		9,000	50% red. photosynthesis		unavailable)
Freshwater Green - Chlorophyceae	> 95	6,900	50% red. cell count	???	Supplemental
Scenedesmus quadricauda		6.500	50% red. Growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		4,000	50% red. photosynthesis		unavailable)
Freshwater Green - Chlorophyceae	> 95	> 10,000	50% red. cell count	???	Supplemental
Chlorella pyrenoidosa		> 10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days[1]; nominal)		3,600	50% red. photosynthesis		unavailable)
Fresh. Blue-Green - Cyanophyceae	> 95	5,500	50% red. cell count	???	Supplemental
Anabaena variabilis		9,200	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		4,700	50 % red. photosynthesis		unavailable)

Degradate Deisopropylatrazine Nontarget Aquatic Plant Toxicity (Tier II)

Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit slope	% Response	MRID No. Author/Year	Study Classification
Fresh. Blue-Green - Cyanophyceae	> 95	> 10,000	50% red. cell count	???	Supplemental
Anabaena cylindrica		> 10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		9,300	50% red. photosynthesis		unavailable)

Degradate Diamino-Atrazine Nontarget Aquatic Plant Toxicity (Tier II)

Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit slope	% Response	MRID No. Author/Year	Study Classification
Fresh. Blue-Green - Cyanophyceae	> 95	7,000	50% red. cell count	???	Supplemental
Anabaena inaequalis		>10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days[1]; nominal)		>100,000	50% red. photosynthesis		unavailable)
Freshwater Green - Chlorophyceae	> 95	4,600	50% red. cell count	???	Supplemental
Scenedesmus quadricauda		10,000	50% red. Growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		>100,000	50% red. photosynthesis		unavailable)
Freshwater Green - Chlorophyceae	> 95	>10,000	50% red. cell count	???	Supplemental
Chlorella pyrenoidosa		>10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days[1]; nominal)		>100,000	50% red. photosynthesis		unavailable)
Fresh. Blue-Green - Cyanophyceae	> 95	>10,000	50% red. cell count	???	Supplemental
Anabaena variabilis		>10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		100,000	50 % red. photosynthesis		unavailable)
Fresh. Blue-Green - Cyanophyceae	> 95	>10,000	50% red. cell count	???	Supplemental
Anabaena cylindrica		>10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		>100,000	50% red. photosynthesis		unavailable)

Degradate Hydroxyatrazine Nontarget Aquatic Plant Toxicity (Tier II)

Species/ Duration/Measured/nominal	% ai	Conc. (ppb) Probit slope	% Response	MRID No. Author/Year	Study Classification
Fresh. Blue-Green - Cyanophyceae	> 95	>10,000	50% red. cell count	???	Supplemental
Anabaena inaequalis		>10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days[1]; nominal)		>100,000	50% red. photosynthesis		unavailable)
Freshwater Green - Chlorophyceae	> 95	>10,000	50% red. cell count	???	Supplemental
Scenedesmus quadricauda		>10,000	50% red. Growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		>100,000	50% red. photosynthesis		unavailable)
Freshwater Green - Chlorophyceae	> 95	>10,000	50% red. cell count	???	Supplemental
Chlorella pyrenoidosa		>10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days[1]; nominal)		>100,000	50% red. photosynthesis		unavailable)
Fresh. Blue-Green - Cyanophyceae	> 95	>10,000	50% red. cell count	???	Supplemental
Anabaena variabilis		>10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		>100,000	50 % red. photosynthesis		unavailable)
Fresh. Blue-Green - Cyanophyceae	> 95	>10,000	50% red. cell count	???	Supplemental
Anabaena cylindrica		>10,000	50% red. growth rate	Stratton 1984	(NOAEC and raw data
(12-14 days; nominal)		>100,000	50% red. photosynthesis		unavailable)

* ??? indicates MRID no. pending

The Tier II results for atrazine degradates indicate that deethylatrazine is more toxic than the other four degradates and the most sensitive algae of the five species usually is the blue-green alga *Anabaena inaequalis* with EC_{50} values ranging from 100 to > 100,000 ppb. Atrazine is more toxic to these algal than any degradate. The order of descending toxicity for these algal species are atrazine > deethylatrazine > deisopropylatrazine > diamino-atrazine > hydroxy-atrazine. The data are useful, but the test species are not the species specified for pesticide registration. The requirement (123-2) has not been fulfilled.

e. Multi-species Tests (Microcosms, Field Studies)

i. Simulated Aquatic Field Studies (Microcosms)

a. Freshwater Microcosm Tests

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o percent difference from controls	Narrative of Study Trends	MRID No. Author/Year
Freshwater microcosm: Measured close to nominal throughout the testing period: concentrations of 0.5, 5, 50, 100, 500, and 5000 ppb	0.5 and 5 ppb o no reduction in net oxygen loss 50 ppb o 25-30% reduction in net oxygen loss 100 ppb o 40-50% reduction in net oxygen loss 500 ppb o 90% reduction in net oxygen loss 5,000 ppb o 100% reduction to negative net oxygen production	*Spirogyra, Oedogonium, Microcystis, Apthanothece, and Scenedesmus* sp. in mixed culture. Microcosms inoculated with algae demonstrated effects at concentrations ≥50 ppb. Physical appearance of the microcosms was altered at 5,000 ppb. Observations and reculture demonstrated that the effects were algistatic.	45087407 Brockway *et al*., 1984
Freshwater Microcosm: (Duration: 7 weeks exposure) Mean measured concentrations of 5.08 ± 0.03 Fg/L; range: 4.2 - 6.0 Fg/L	NOEC: 5 ppb o slight non-sign. shifts in water parameters: o DO decreased from means of 9.4 - 9.9 mg/L (controls) differing weekly by 0.2 - 0.6 mg/L o pH decreased from means of 8.4 - 9.0 (controls) differing weekly by 0.0 - 0.4 units o conductivity increased from 159.3 - 189.3 FS/cm (controls) differing by 0.2 - 10.0 FS/cm o alkalinity increased from means of 1.4 - 2.2 mg/L (controls) differing by 0.0 - 0.3 mg/L o no significant adverse effects on phyto- & zooplankton, or 15 macro-invertebrate species o Cyclopoida sign. increased in week 3	Laboratory microcosms (4 replicates) were tested with 0 and 5 Fg/L atrazine for 7 weeks. The plankton and macro-invertebrates were introduced together with 2-cm layer of natural sediments into glass aquaria with a 50 cm water column with a 14-hour photoperiod. Water was circulated through the microcosms at a flow rate of 3.5 L/min. during an acclimation period for biota of 3 months. This test was part of a study of pesticide interaction between atrazine and chlorpyrifos to determine the adequacy of chronic safety factors. The authors concluded that the slightly changes in water parameter found with atrazine indicates a small effect decreasing photosynthesis.	45087417 van den Brink *et al*. 1995 Supplemntal (raw data unavailable)
Freshwater Microcosm: Duration: 21 days Mean measured conc. of 3.2, 10, 32, 110, and 337 ppb	NOEC: 10 ppb: sign. incr. protein (60%) / chlorophyll (78%) LOEC: 32 ppb: o sign. reduced DO (35%), Mg (18%), & Ca (16%) o sign. red. microbial pop.(40%) on Day 3 o sign. incr. microbial pop. Days 14 (25%) & 21 (61%) 110 ppb: o sign red. microbial pop. (60%) on Day 3; recovered o sign. red. DO (32%) and magnesium (10%) 337 ppb: o sign. red. microbial pop. on Days 3 (45%), 14 (31%), & 21 (23%) o sign. red. protein (41%), chlorophyll (91%), DO (27%), K (44%), Mg (21%), & Ca (17%)	Laboratory microcosms were inoculated with foam blocks which were colonized in a pond for 14 days. The effect to protozoans from atrazine exposure was examined by measuring structure (species number, biomass), and function (colonization rate, oxygen production, chlorophyll concentration) of the community as well as K, Mg and Ca ion concentrations of the biomass after 21 days. Microbial populations recovered by Day 21 at all test levels except 337 Fg/L. Levels of significance difference are ($p < 0.05$).	45087416 Pratt *et al*. 1988 Supplemental (raw data unavailable)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o percent difference from controls	Narrative of Study Trends	MRID No. Author/Year
Freshwater Microcosm: (6 weeks) Meas. peak 20 ppb on day 1, mean measured concentration of approximately 10 ppb	10 ppb (6 weeks) o sign. (0.05) reduced dissolved oxygen (DO), but was recovering by test termination	Laboratory microcosms were treated with a stock solution of atrazine and soil to which atrazine was bound. At the end of the study, no significant effects on plant biomass or daphnid/midge survival were noted, but DO was affected.	45205102 Huckins *et al*. 1986 Supplemental (raw data unavailable)
Freshwater microcosm: (30 days): Macrophytes, algae, zooplankton and benthic invertebrates; Nominal conc. of 10, 100 and 1,000 ppb as a soil slurry	10 ppb (Day 2) o 23% red. in gross primary productivity (GPP); recovery by Day 7 and similar to controls at Day 30 100 ppb (Day 2) o 32% red. in GPP; recovery by Day 7 and similar to controls at Day 30 1,000 ppb (Day 2) o 91% red. in GPP; no recovery, 70% red. throughout test 1,000 ppb (Day 30) o 48% red. (sign. P≤0.05 level) macrophyte biomass o 36% red. (sign., P≤0.05) *Selenastrum* dry weight 1,000 ppb (30-day aged microcosm water) o 76% red. (sign. P≤0.05) *Selenastrum* dry weight 1,000 ppb (Day 30) o reduced O_2, community respiration, pH o 20% increase in conductivity o 120% increase in alkalinity o no effect on soil microbial activity	4-L microcosms were established in the laboratory and treated with a soil slurry of atrazine. The endpoints examined over the 30-day experiment included effects to zoo- and phytoplankton as well as macrophytes (i.e., *Lemna* sp., *Ceratophyllum* sp., and *Elodea* sp.). Static acute and chronic assays were conducted with *Daphnia magna* and *Chironomus riparius* using treated water that had come from the microcosm after 30 days or from a vessel that contained the treated water for 30 days (i.e., aged treated water). The author concluded that microcosm itself ameliorated the phytotoxic effect at 1,000 ppb. No effect on invertebrates up to 1,000 ppb and effects to phytoplankton at 10 and 100 ppb were not observed by test termination (30 days). Conductivity, pH, and alkalinity were also affected at 1,000 ppb.	45087413 Johnson, 1986 Supplemental (raw data unavailable)
Freshwater Microcosm: Emergent vascular plants; Nominal water conc. of 10, 50, 100, 500, and 1,500 ppb; measured water conc. in the 50 and 500 ppb treatments of 1.3 and 1.6 ppb, respectively, after 16 weeks	500 ppb (6 weeks) o sign. (0.05 level) red. shoot length of *Scirpus acutus* 1,500 ppb (6 weeks) o sign. red. shoot length of *Scirpus acutus* and *Typha latifolia*	Greenhouse microcosms were made by placing rhizome sections in tubs which were filled with treated water to 1 cm above the soil surface. The plants were allowed to grow for 16 weeks and shoot height of hardstem bulrush and broad-leaved cattail was monitored bi-weekly. Also non-sign. effects of chlorosis and reduced growth noted at 50 and 100 ppb. A second test demonstrated resiliency of both plants at 500 ppb.	45087415 Langan and Hoagland, 1996 Supplemental (raw data unavailable)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o percent difference from controls	Narrative of Study Trends	MRID No. Author/Year
Freshwater Microcosm: (14 days) Measured atrazine concentrations approximately 75% of nominal (15 and 153 ppb) for first application and 150% of nominal (385 and 2,167 ppb) for the second application	Sign. (0.1 level) reduction in turbidity and chlorophyll (7 days), and increase in phosphorous (day 14) and nitrogen (days 7 and 14) after the 1st application. Copepod and rotifer densities were also sign. reduced on days 7 and 14. Sign. reductions in productivity, chlorophyll, green algal colonies, rotifers, and *Bosmina* sp. (zooplankton) after 2nd application. Phosphorous, nitrogen, and pH were also sig. affected.	A 3x3 factorial design with three conc. of atrazine (0, 15, and 153 ppb) and three conc. of bifenthrin (0, 0.039, and 0.287 ppb) applied as soil slurry in May, then again one month later but with atrazine conc. of 0, 385, and 2,167 ppb and bifenthrin conc. of 0, 0.125, and 3.15 ppb. Atrazine alone caused dose-responsive reductions in chlorophyll, turbidity, primary production, increases in nitrogen and phosphorous, and reduced levels of chlorophytes, cladocerans, copepod nauplii, and rotifers. General recovery after 14 days for atrazine alone in the first phase, but recovery not complete at sampling termination after second phase (14 days). No synergistic or antagonistic effects were noted.	45020014 Hoagland *et al*., 1993 Supplemental (raw data unavailable)
Freshwater microcosm: (2 months; measured) Nominal concentrations of 0, 60, 100, 200, 500, 1,000 and 5,000 ppb. Measurements made three times during the two month study.	60 ppb (nominal) o 14-carbon uptake decreased immediately after treatment; recovery began after 10 days; o stimulated production of chlorophyll a; 100 ppb (nominal) o 14-carbon uptake decreased immediately after treatment; recovery began after 10 days; o stimulated production of chlorophyll a; 200 ppb (nominal) o 14-carbon uptake decreased immediately after treatment; slight recovery 2 months after treatment; o stimulated production of chlorophyll a; o inhibited increases in dissolved oxygen during light phase and decreases in DO during dark phase 500 ppb (nominal) o 14-carbon uptake decreased immediately after treatment; no recovery; o minimal inhibition of chlorophyll a production; 1,000 and 5,000 ppb (nominal) o 14-carbon uptake decreased immediately after treatment; recovery began after 10 days. EC50s for Days 0-10, 53-60, & Mean (mean measured conc.) Time period; 14C uptake; DO (light); DO (dark) Days 0-10 : 103 ppb 126 ppb 106 ppb Days 53-60: 159 ppb 154 ppb 164 ppb Days 1-60: 131 ppb 165 ppb 142 ppb	Results of single species assays, microcosm, and pond studies were compared. 14-Carbon fixation was used as the end-point for all three study types. Laboratory results with eight algal species ranged from 37 to 308 ppb for carbon uptake inhibition EC_{50} values. Microcosm EC_{50} values ranged from 103 to 159 ppb. The mean pond EC_{50} was 100 ppb for carbon uptake and 82 ppb for chlorophyll-a inhibition. The authors stated that multiple laboratory studies or a microcosm study represent(s) entire ecosystem functional effects.	45020015 Larsen *et al*., 1986 and 45087419 Stay *et al*. 1985 Supplemental (raw data unavailable)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o percent difference from controls	Narrative of Study Trends	MRID No. Author/Year
Freshwater microcosm: (60 days; measured) Nominal concentrations of 60, 100, 200, 500, 1,000, and 5,000 ppb. Concentrations measured on Days 7, 28, 53, 60.	NOEC < 60 ppb; 60 ppb (1 - 20 days) o sign. (0.05) red. 14-carbon uptake for first 20 days ≥ 100 ppb (2 weeks) o sign. (0.05 level) red. primary productivity; o sign. red. in productivity/ dark respiration ratio; o pH sign. less than control values ≥ 500 ppb (6 weeks) o all endpoints declined immediately after treatment and never recovered during the experiment.	Taub microcosms were 3-L jars inoculated with 10 algal species on Day 0, *Daphnia magna* and 4 other animal species on Day 4. On Day 7, 27 microcosms were treated with atrazine; no other atrazine treatments um from four different aquatic systems. Community metabolism was measured for primary productivity and light and dark respiration. At the high treatment levels (500, 1000 and 5000 ug/L), all process variables declined immediately after atrazine treatment and did not recover during the experiment. At the low treatment levels (60, 100 and 200 ug/L), the magnitude of the responses to atrazine was not constant, but with 3 phases; an autotrophic phase, daphnid bloom and an equilibrium phase.	45087419 Stay *et al*., 1989 Supplemental (raw data unavailable)
Freshwater microcosm: (6 weeks; measured) Single dose; Nominal conc. 20, 100, 200, 500, 1,000 and 5,000 ppb. Concentrations were measured on Days 0 and 42. On Day 42, atrazine levels averaged 69 to 80% of the initial concentrations.	NOEC = 20 ppb LOEC = 100 ppb in 3 out of 4 natural plankton communities and 200 ppb for the fourth community. ≥ 100 ppb (2 weeks) o sign. (0.05 level) red. primary productivity o sign. red. in productivity/dark respiration ratio o pH sign. less than control values	Leffler microcosms were constructed with inoculum from four different aquatic systems from natural communities and contains organisms representing several trophic levels. The vessels were dosed after 6 weeks of seeding and monitoring for 6 more weeks. The LOEC for 3 of the systems was reported to be 100 ppb, while the LOEC for the fourth was 200 ppb.	45087418 Stay *et al*. 1989 Supplemental (raw data unavailable)

b. Marine/Estuarine Microcosm Tests

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o percent difference from controls	Narrative of Study Trends	MRID No. Author/Year
Estuarine microcosm: (; nominal) Wild Celery *Vallisneria americana* 1 treatment Nominal concentrations of 4, 8, 16, 32, and 64 ppb	NOEC < 4 ppb 4 ppb (reproductive season) o sign. 16% reduction in tuber formation o 55% reduction in biomass 8 ppb (reproductive season) o 21% reduction in tuber formation 16 ppb (mid season and reproductive season) o 60% reduction in tuber formation o 27% reduction in tuber weight o sign. reduction in leaf growth, biomass, and female flowers 64 ppb (reproductive season) o 75% reduction in tubers o red. female flowers	Laboratory microcosms were used to grow *Vallisneria americana* through entire seasons (divided into three periods - early-, mid-, and reproductive). The aquaria were dosed one time at the nominal conc. after a 14-day acclimation period. With respect to leaf growth, atrazine caused the plants to be shorter and more fragile. With respect to flowering and rhizome production, effects were generally first noted at the 16 to 32 ppb range. Tuber formation appeared to be the most sensitive endpoint, with production in terms of numbers significantly reduced at the 4 ppb level.	45020001 Cohn 1985 Supplemental (raw data unavailable)
Estuarine lab. microcosm: (7-day exposure; nominal) Nominal concentrations of 22, 220, and 2,200 ppb Estuarine field microcosm: (108-days; nominal) Single exposure; Nominal applications of 0.4, 1.4, 4.5, and 45 lb ai/A	"NOEC" = 10 ppb (based on author's use of a 10-fold safety factor from the I_1 level = 100 ppb) 220 ppb (1 week) o sign. (0.05 level) red. in cell # of *Thalassiosira fluviatilis* o sign. red. in photosynthesis of *T. fluviatilis* and *Nitzschia sigma* 2,200 ppb (1 week) o sign. red. in cell #, photosynthesis., and chlorophyll content for both algae 1.4 lb ai/A (effect up to 5 days) o sign. red. in surface chlorophyll and primary prod. (85-89%) 1.4 lb ai/A (effect up to 8 & 17 days) o sign. reduction in carbon fixation (52-73%) 0.4/4.5 lb ai/A (effect at 16 days, but not 26 days) o sign. reduction in carbon fixation 45 lb ai/A (42 days) o sign. red. in carbon fixation	Laboratory studies were conducted with the salt marsh edaphic diatoms *Thalassiosira fluviatilis* and *Nitzschia sigma*. The I_{50} for both species combined was reported to be 939 ppb. The I_1 was reported to be 100 ppb, and by applying a 10-fold safety factor, the acceptable level (NOEC) was reported to be 10 ppb. Subsequently, studies were conducted in greenhouse microcosms (1.4 lb ai/A) and in two field studies (1.4 lb ai/A or 0.4, 4.5, and 45 lb ai/A) on the beach wherein enclosures were sunk into the sand and exposed to tidal action. Atrazine treatment also appeared to cause a shift to a *Navicula* sp. dominated system. Field results with higher rates of atrazine were as expected, with carbon fixation reduced for up to 16 days at the 2 lower rates and up to 42 days at the highest rate.	45087406 Plumley and Davis, 1980 Supplemental (raw data unavailable)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o percent difference from controls	Narrative of Study Trends	MRID No. Author/Year
Estuarine microcosm: (5 Weeks; measured) 3 weekly applications followed by 2 weeks observation. Mean measured concen. at approximately mid-point of the *Spartina* test were 30, 280, and 3,100 ppb and in the *Juncus* test were 30, 250 and 3,800 ppb.	30, 280, and 3,100 ppb (5 weeks) o sign. (0.05 level) increase in peroxidase activity in *Spartina alterniflora* 30, 250, and 3,800 ppb (5 weeks) o sign. (0.05 level) red. in chlorophyll-a (Chl-a) and Chl-a/Chl-b ratio in *Juncus roemerianus* 250 and 3,800 ppb (5 weeks) o sign. red. in Chl-b in *J. roemerianus* 3,100 ppb (1 week) o sign. red. in growth in *S. alterniflora* 3,800 ppb (5 weeks) o sign. red. in growth in *J. roemerianus* o sign. increase in oxidized lipids in *J. roemerianus* 250 ppb (1 year) o partial recovery in *J. roemerianus* 3,800 ppb (1 year) o practically no survival of *J. roemerianus*.	Two aquatic estuarine plants were exposed to atrazine in greenhouse microcosms. The plants were exposed to atrazine by placing treated sand on the surface of the pots three times (once a week for the first 3 weeks of the study) followed by 2 more weeks for a total of 5 weeks. The water samples were taken after the third application. The pots were also tidally-exposed (i.e., low tide during the day and high tide at night). *S. alterniflora* plants demonstrated a dose-response increase in peroxidase activity. In contrast, *J. roemerianus* plants demonstrated a dose-responsive reduction in chlorophyll and an increase in the amount of oxidized lipids. The authors state that atrazine "should pose no significant adverse effects on *S. alterniflora*. In contrast, if chronic levels of atrazine persist in the range of 250 ug/L or greater, *J. roemerianus* most likely will exhibit die off or decline that may lead to loss of this species within the habitat."	45087405 Lytle and Lytle, 1998 Supplemental (raw data unavailable)
Estuarine microcosm: (unk;) Nominal concentrations of 0, 50, and 100 ppb	Both *Nannochloris oculata* and *Phaeodactylum tricornutum* were significantly (mostly at the 0.01 level) affected by changes in light, temperature, and atrazine conc.	A 3x3x3 factorial design examined the effect of temperature, light, and atrazine conc. on two species of estuarine algae. *N. oculata* was sig. affected by all variables, and the three two-way and one three-way interactions were also significant. *P. tricornutum* was affected by the main variables and the only sig. interaction was light by atrazine.	Mayasich *et al*., 1986
Estuarine microcosm: (unk) Nominal concentrations of 0, 15, 30, and 50 ppb	The above mentioned algae were tested together and this variable also caused a sig. (0.01 level) effect on *N. oculata* growth rate	An extension of the above described study. In addition to separate culture, the two estuarine algae were cultured together. The end result was that *P. tricornutum* dominated the cultures due to the stress of atrazine to *N. oculata* under optimum growth conditions	Mayasich *et al*., 1987

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o percent difference from controls	Narrative of Study Trends	MRID No. Author/Year
Estuarine microcosm: (4 weeks; measured) Mean measured concentrations in water were 130 ppb for the "low" treatment and 1,200 ppb for the "high" treatment over a four week period (4 weeks; measured)	130 ppb (Week 1) o no photosynthesis 130 ppb (Week 2-4) o sign. red. in plant total biomass; no change in biomass for 3 weeks 130 ppb (Weeks 1-4) o sign.; averaged 50% red. photosynthesis of *Potamogeton perfoliatus* during the test; steady recovery after first week, but not fully recovered 1,200 ppb (Weeks 1-4) o sign.; 100% red. photosynthesis throughout the test 1,200 ppb (Weeks 2-4) o sign.; plant total biomass steadily reduced 1,200 ppb (Weeks 3-4) o sign.; 80% red. shoot density	Aquatic plants were planted and atrazine-treated sediments were added to 700-L microcosms. On Day 1.5, 93.4% of the total atrazine was dissolved in water. In addition to photosynthesis, it was demonstrated that shoot growth was relatively unaffected at 130 ppb, but total biomass was sign. reduced after 2-4 weeks. Plant biomass reductions followed a 1 week lag after photosynthesis reduction. At 1,200 ppb, plant biomass had been virtually eliminated by the end of the test. Mean shoot length in the controls declined after week 1 and after week 3 for 1,200 ppb.	45087403 Cunningham *et al*., 1984 Supplemental (raw data unavailable)
Estuarine microcosm: (22-23 days; measured) Single dose: Day 0: 30,000 ppb - nominal; Measured only Day 22 or 23: 16,400-17,700 ppb	30,000 ppb (Day 5-22) o sign. ($p \leq 0.05$) red. average ratio of no. of ramets (branches): initial no. of ramets 30,000 ppb (Day 22 or23) o sign. ($p \leq 0.05$) 46-58% red. in total above-ground biomass o sign. ($p \leq 0.05$) 18% red. in average dry weight per ramet	Experiments were conducted with seagrass, *Halodule wrightii*, examining the effect of atrazine and any interactions of salinity (15, 25, 35 ppt), light intensity (115, 140, 173 $uEm^{-2}s^{-1}$), and cropping (either cut at 4-cm or 6-cm). None of these environmental factors affected the response of the grass to atrazine.	45205101 Mitchell, 1987 Supplemental (raw data unavailable)

ii. Aquatic Field Studies (including Mesocosms and Limnocorrals)

a. Freshwater Ponds, Lakes and Reservoirs

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Freshwater Lake: Plankton (Duration 18 days) Measured = ≥90% of nominal over the test period (18 days): nominal concentrations of 0.1, 1, 10, and 100 ppb	NOEC = < 0.1 ppb o transient effects on water chemistry 1 ppb (1 week) o decreased primary production; o increased bacterial numbers o decreased in zooplankton numbers (cladocerans affected greater than copepods) 10 ppb (3 weeks) o 65% sign. ($p < 0.01$) red. in daphnid population growth (combined effect of water & algae) o 59% sign. ($p < 0.05$) red. in daphnid growth (algae) 100 ppb (3 weeks) o 92% sign. ($p < 0.01$) red. in daphnid growth (combined) o 69% sign. ($p < 0.01$) red. daphnid growth (algae)	*In situ* enclosures in a German lake were treated and monitored over 18 days. Dose-responsive reductions in chlorophyll-a and oxygen and increases in particulate organic carbon were observed at 1, 10, and 100 ppb. Within 1 week at 1 ppb, primary production decreases and bacterial number increases were observed. Zooplankton numbers then decreased, with cladocerans affected more than copepods. Additional studies at 0.1 ppb also demonstrated transient effects on water chemistry and biological parameters. Most of the parameters were recovered or were recovering within 42 days of application.	45087414 Lampert *et al*., 1989 Supplemental (raw data unavailable)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Freshwater Pond: Plankton Treated 3 times on 7/31, 8/28 (29 days later), and 9/21/1990 (24 days later) at 5, 10, 25, 75, 200, and 360 ppb. Weekly conc. relatively constant; mean measured conc. over two months are 5, 10, 22, 68, 182, and 318 ppb (63 days; measured)	NOEC: 5 ppb (63 days) compared to controls 10, 22 and 68 ppb o up to 40% red. dissolved oxygen (Days 7-46) o up to 10% incr. pH (Days 18-63) o up to 10% red. conductivity (Days 7-53) 68 ppb o up to 78% red. copepod nauplii and no increase in nauplii at 182 & 318 ppb o diatoms appear to become the dominant phytoplankton 182 ppb o strong red. in dissolved oxygen and conductivity and strong increase in pH levels (same for 318 ppb) o up to 98% red. Cryptophyceae, *Cryptomonas marsonii* and *S. erosa/ovatata* (Days 21 to tests end) o up to 10% red. conductivity (Days 7-53) o up to 98% red. seasonal blooms of *Cryptomonas marsonii* & *S. erosa/ovatata* (Days 21 to tests end) o prevented *Mallomonas* sp. seasnal bloom (318 ppb too) o prevented the seasonal bloom of *Planktosphaeria* sp. (Chlorophyceae) after Day 30 (same at 318 ppb) o lower numbers & early seasonal decline of rotifers, *Synchaeta* sp. (same at 318 ppb) 318 ppb o up to 80% red. phytoplankton cell density (throughout test, except on Day 35) o up to 98% red. Cryptophyceae, *Cryptomonas marsonii* and *S. erosa/ovatata* (first appeared on Day 10 - Days 21 to tests end) o up to 9% incr. pH (Days 18-63) o up to 10% red. conductivity (Days 7-53) o strong red. in cell numbers of *Planktosphaeria* sp. (Chlorophyceae) after Day 30 o delays in reaching and lower peak daphnid egg ratio, and delayed peaks for numbers of young and adults	Mesocosms (1,000 L cylinders) in southern Bavaria were treated with atrazine 3 times (29 and 24 day intervals) over 63 summer days. Strongly dose-response reductions in dissolved O_2, pH, and conductivity were noted at concentrations greater than 5 ppb. Changes in oxygen concentrations at $\geq$ 10 ppb and some zooplankton populations at 68, 182, and 318 ppb reflect indirect functional links as a result of altered primary production. At 68 ppb, up to a 78% reduction in copepod nauplii was found and no increase in the number of nauplii was found at 182 and 318 ppb. At 182 ppb, threshold concentrations for direct effects by atrazine were exceeded in several phytoplankton species. Diatoms appeared to become the dominant phytoplankton at 182 and 318 ppb. One rotifer species decreased at 182 ppb and another at 318 ppb and was virtually absent from Day 18 to the end of the study. Daphnid reproduction and populations decreased at 318 ppb.	45020022 Juttner *et al*. 1995 Supplemental (raw data unavailable)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Freshwater Ponds: Measured in the water column four times during the first two months of the study: 100% of nominal at time zero (nom. conc. of 20 or 500 ppb) (163 days; measured)	Laboratory data shows results for atrazine sensitivity tests for treated field samples: 1 ppb o sign. (0.05) 4% increase in fluorescence 5 ppb o sign. (0.05) 9% increase in fluorescence o sign. (0.05) 8% decrease in C-14 uptake 20 ppb o sign. (0.05) 30% increase in fluorescence o sign. (0.05) 12% decrease in C-14 uptake 500 ppb o sign. (0.05) 136% increase in fluorescence o sign. (0.05) 88% decrease in C-14 uptake Field pond study results: 20 ppb o sign. (0.05) 51% red. C-14 uptake (4 hr.) (Days 2-7) o sign. 42% red. phytoplankton biomass (Days 2-7) o 3% red. growth & 28% red. daphnid reproduction *Simocephalus serrulatus* correlated with food levels 500 ppb o pH red. 0.3 units lower than controls for a few weeks o dissolved O_2 generally red. 1-3 mg/L (a few weeks) o sign. 94% red. C-14 uptake (4 hr.) (Days 2-163) o usually sign. red. phytoplankton biomass (Days 2-136) o rapid, nearly complete red. in the abundant *Peridinium inconspicuum*, a small dinoflagellate and rapid red. in 7+ other dominate phytoplankton sp. after 7 days o incr. in several flagellate species; mainly *Mallomonas pseudocoronata*, *Cryptomonas marssonii* & *C. erosa* o zooplankton dominance shifted to rotifers, mainly *Keratella cochlearis* after Day 31 o >50% red. in the copepod, *Tropocyclops prasinus mexicanus* by Day 14	Single treatment of two 0.045 hectare ponds each with either 20 or 500 ppb atrazine produced dose responsive changes in pH, DO and daily carbon uptake. Phytoplankton growth was reduced; population shifts were apparent at 20 and 500 ppb. Effects on phytoplankton were immediate, within 2 days, for daily carbon-14 uptake and biomass declines at both treatment levels, which is consistent with other researchers in laboratory tests. Atrazine concentrations down to 1 ppb affected photosynthesis in lab tests with phytoplankton samples from the pond. While atrazine produced direct toxic effects on just certain members of the aquatic community, their responses also affected other members of the community. At 500 ppb, one species of herbivorous zooplankton declined by more than 75% within 14 days of treatment. Subsequent laboratory tests demonstrated some atrazine resistance in phytoplankton and showed zooplankton population effects were due to loss of food (algae). Further evidence of resistance was indicated by a dominant phytoplankton species which showed less toxic responses than the same species in the control pond. The authors concluded that lab studies of other researchers with isolated phytoplankton species correctly predict the existence and immediate severity of the effects of atrazine, but those study do not indicate the indirect effects seen on other species in the aquatic community.	45020011 DeNoyelles *et al*. 1982 Supplemental (raw data unavailable)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Artificial freshwater ponds in Kansas treated with atrazine to achieve concentrations of 20 and 500 Fg/L	NOAEC < 20 Fg/L 20 Fg/L - 60% sign. ($p < 0.05$) reduction in macrophtye vegetation at summer's end including elimination of *Potamogeton pusillus, P. nodosus*, & *Najas quadalupensis*; - 95% sign. ($p < 0.05$) red. macrophyte coverage in May, 10 months after treatment; - 96% sign. ($p <0.01$) reduction in the number of young bluegill; - 85% sign. ($p < 0.001$) red. in the number of food items/ fish stomach; - 57% sign. ($p < 0.001$) red. in the number of prey taxa/ fish stomach. 500 Fg/L - 90% sign. ($p < 0.05$) reduction in macrophtye vegetation at summer's end including elimination of *Potamogeton pusillus, P. nodosus*, & *Najas quadalupensis*; - >95% sign. ($p < 0.05$) red. macrophyte coverage in May, 10 months after treatment; - 96% sign. ($p <0.01$) reduction in the number of young bluegill; - 78% sign. ($p < 0.001$) red. in the number of food items/ fish stomach; - 52% sign. ($p < 0.001$) red. in the number of prey taxa/ fish stomach.	Two artificial Kansas ponds each (0.045 ha. and 2.1 m. deep) were treated with 20 Fg/L and 500 Fg/L on 24 July and two ponds served as controls. The macrophyte community in treated ponds was noticeably reduced, relative to controls, throughout the summer. Visual estimates of the macrophyte communities in the ponds showed roughly a 60 percent decline in the 20 Fg/L ponds and a 90 percent decline in the 500 Fg/L ponds two months after atrazine addition. These estimates were verified by rake hauls which produced these same relative differences. The following May, 10 months after treatment, when macrophytes are normally well established in Kansas ponds, the ponds were drained. Relative to control ponds, 20 Fg/L ponds had a 90 percent reduction in macrophyte coverage and the 500 Fg/L ponds had a >95 percent reduction in macrophyte coverage. Differences were noted in the macrophyte species present. Control ponds contained *Potamogeton pusillus* and *P. nodosus, Najas quadalupensis*, and small amounts of *Chara globularis*, whereas the treated ponds contained mostly *C. globularis*. Significant indirect effects were found on bluegill diet and reproduction.	45202912 Kettle, de Noyelles, Jr., Heacock and Kadoum 1987 Supplemental (raw data are not available for analyses)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Freshwater limnocorrals: (3 controls and 3 treated at nominal concentrations of 100 ppb on June 1 & July 6, 1983) Measured conc. range: 80-140 ppb after the first application, 120-165 ppb after the second application (329 days; measured)	Effects on periphyton and environmental parameters: first application: 80 - 140 ppb o no sign. effects on DO, temperature, Secchi depth, dissolved inorganic carbon (DIS), NO_3-NO_2-N), total nitrogen, and total phosphorus o periphyton dry wt. lower than controls after Day 14 at most depths; sign. (0.05) red. at a depth of 0.5 m on Day 34 and thereafter o sign. 94% red. C-14 uptake (4 hr.) (Days 2-163) o usually sign. red. phytoplankton biomass (Days 2-136) o rapid, nearly complete red. in the abundant *Peridinium inconspicuum*, a small dinoflagellate and rapid red. in 7+ other dominate phytoplankton sp. after 7 days o incr. in several flagellate species; mainly *Mallomonas pseudocoronata*, *Cryptomonas marssonii* & *C. erosa* o zooplankton dominance shifted to rotifers, mainly *Keratella cochlearis* after Day 31 o >50% red. in the copepod, *Tropocyclops prasinus mexicanus* by Day 14 second application 120 - 165 ppb o sign. (0.05) 20% red. dissolved oxygen (Days 37-137) o sign. (0.05) 33% increase in Secchi depth o sign. (0.05) 62% increase dissolved inorganic carbon o sign. (0.05) 103% increase in NO_3-NO_2-N o sign. (0.05) red. periphyton dry weight at depths of 0.5 and 1.5 m on most sampling days o sign. (0.05) red. decr. chlorophyll (19 days after second appl. (Day 54 & on some days thereafter) o zooplankton dominance shifted to rotifers, mainly *Keratella cochlearis* after Day 31 o >50% red. in the copepod, *Tropocyclops prasinus mexicanus* by Day 14	Elaboration of the 80 ppb treatment from Hamilton *et al*., 1987. After the first application (pulse), blue-green algae were eliminated and organic matter was significantly reduced. After the second pulse, organic matter, chlorophyll, biomass, and carbon assimilation were reduced by between 36 and 67%, along with certain species of green algae. Diatom numbers were greater in treatment limnocorrals than in the control limnocorrals for nine weeks after the second pulse.	45020012 Herman *et al*., 1986 Supplemental (raw data unavailable)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Texas Lake Mesocosm: Measured atrazine concentrations approximately 75% of nominal (15 and 153 ppb) for first application and 150% of nominal (385 and 2,167 ppb) for the second application	Phyto- and zooplankton	A 3x3 factorial design with three conc. of atrazine (0, 15, and 153 ppb) and three conc. of bifenthrin (0, 0.039, and 0.287 ppb) applied as soil slurry in May, then again one month later but with atrazine conc. of 0, 385, and 2,167 ppb and bifenthrin conc. of 0, 0.125, and 3.15 ppb. Atrazine alone caused dose-responsive reductions in chlorophyll, turbidity, primary production, increases in nitrogen and phosphorous, and reduced levels of chlorophytes, cladocerans, copepod nauplii, and rotifers. General recovery after 14 days for atrazine alone in the first phase, but recovery not complete at sampling termination after second phase (14 days). No synergistic or antagonistic effects noted.	45020014 Hoagland *et al*., 1993 Supplemental (raw data unavailable) Duplicate check & delete this
Artificial ponds: (unk); measured Mean measured concentrations of 18.4, 91.5 or 114 ppb (two years data), and 314 ppb	Aquatic plants, phyto- and zooplankton	Nominal applications of either 20, 100, or 300 ppb atrazine were monitored for effect 8 weeks after June application and in the next summer. Conductivity and oxygen concentration were affected at the 100 and 300 ppb levels. Reductions in aquatic plant numbers were observed at $\geq$100 ppb in the summer after application, but no effects on microflora or fauna were observed. The year after treatment (with 10 to 30% of atrazine still in the water column), *Chara* sp. replaced *Myriophyllum spicatum* and *Potamogeton natans* at levels $\geq$100 ppb. Phytoplankton became dominated with cyanophytes and then cryptophytes as the concentration of atrazine increased. Zooplankton numbers at 100 and 300 ppb were also reduced the following year.	45020017 Neugebaur *et al*., 1990 Supplemental (raw data unavailable)

b. Freshwater Natural and Artificial Streams

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Small Canadian first-order stream adjacent to a tiled-corn field. Atrazine of unspecified purity was applied at 4 liters per hectare on 6 June 1989. The Canadian Water Quality Guidelines (CCREM, 1987) specify a guideline of 2.0 Fg/L to protect freshwater life.	Non-statistical pair-wise comparison of Total Phytoplankton counts vs sta 9, the control indicates reductions at all downstream stations with effects generally decreasing with time and distance. Downstream station 11 (2.5 km from atrazine source -sta. 5): 0.047 Fg/L (range 0.004-0.2Fg/L) atrazine conc. o all samples with reduced total phytoplankton counts o mean reduction of 63 % (range 6 - 97 %) o highest red. (97 %) on June 9, first sampling day o reduced 70 % in final sample on 16 Nov. Downstream station 10 (50 to 75 m from sta. 5) 0.366 Fg/L (range 0.1 - 1.7 Fg/L) atrazine conc. o 2 out of 11 samples exceed count at sta. 9 o mean reduction of 45 % (range +55 - 92 %) o highest red. (92 %) on June 9 o reduced 47 % in final sample on 16 Nov. Downstream stations 6 & 7 (a few meters from sta. 5) 0.81 (0.17 - 1.89) and 0.05 (0.001-0.224) Fg/L, resp. o 1 out of 9 samples at sta. 6 exceeds count at sta. 9 o mean reduction sta. 6 of 53 % (range +68 - 99) o mean reduction sta. 7 of 66 % (range 3 - 95) o highest red. (99 and 93 %, resp.) on July 21 o red. 45 & 27 %, resp. in final sample on 16 Nov. Ditch (station 5) receiving waters from the 4 tile outlets: 2.62 Fg/L (range 0.211 - 13.9 Fg/L) atrazine conc. o mean reduction of 79 % (range 46 - 99 %) o highest red. (92 %) on 3 dates, June 23 - July 21 o reduced 51 % in final sample on 16 Nov.	Atrazine concentrations up to 20.39 Fg/L (sta. 4) in field tile water, 13.9 Fg/L (sta. 5) in receiving ditch and 1.89 Fg/L in a small stream (sta. 6) were measured in New Brunswick, Canada in a rural headwater basin of the Petitcodiac River. The fist-order stream flowed parallel to an 8-hectare sub-surface tile-drained field of silage corn. The field was divided into 4 plots and each drained separately into a small canal and into the stream. Water, phytoplankton and zooplankton were sampled at 15-day intervals at 11 sampling sites during the growing season. Total phytoplankton numbers in downstream samples were consistently much less than those from upstream (control) samples during the period of low flow and higher atrazine levels (during the summer). Diatoms dominated the phytoplankton community. Occurrence of other algal species were erratic between stations and over time. Zooplankton numbers were too low to discern trends, but downstream samples were consistently lower in individuals than control samples.	45020008 Lakshinarayana, O'Neill, Johnnavithula, Leger and Milburn 1992 Supplemental (Replication of samples and statistical analyses were not made)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Artificial stream test: (14 day; measured) Simulated pulsed- exposures; 5 Fg/l atrazine on Day 1 and gradually diluted until only about 1 Fg/L on Day 7	5 Fg/L to about 1 Fg/L on Day 7 o atrazine concentrations: Day Mean conc. 1 4.74 5 3.56 10 1.20 14 1.19 Possible atrazine effect: o 58 to 126 fold increase sign. ($p<0.05$) in number of emergent insects on Days 3, 5 and 7; treatment numbers were equal to or greater than controls in all samples No statistical effects found in atrazine treatments on: o periphyton growth measured as chlorophyll a levels; chlorophyll a levels decreased gradually in all samples (treatments & controls) over time, "may have masked an effect of atrazine" o indirect effects on function or taxonomic composition of benthic community structure	A community of benthic, stream invertebrates from the Patrick Brook in Hinesburg, Vermont, located in the LaPlatte River watershed. Microbial community growth was incubated for 2 weeks this substrate was placed in 10 x 10 x 7 cm polyethylene boxes and placed in the stream for invertebrate colonization for 3 weeks in July 1993. During the same 3-week period glass slides were placed in the stream for algal settling and growth. Four benthic invertebrate boxes and 9 periphyton slides were randomly placed in each of six replicate tanks. The flow rate was calculated as 20.8 L/min. throughout the test. After a 24-hour equilibration period, treatment at 5 Fg/L atrazine was introduced to 3 replicates and 3 controls. On Day 3, about 15 percent of the water was replaced; on Days 6 and 7 water replacements were 50 percent each day; about 15 % was replaced on Day 11 during the 14-day test. "Dewey (1986) also observed herbivorous insects emerging earlier from artificial ponds treated with 20 Fg/L atrazine compared to controls. Dewey suggested that the changes she saw were the indirect effect of atrazine exposure, which had reduced the amount of food available to herbivorous insects."	45087411 Gruessner and Watzin 1996 Supplemental (raw data unavailable for statistical analyses)
Artificial stream tests: (14 day; measured) One dose and recirculation; two atrazine levels (40.8% ai): 15.2 $\pm$ 1.4 and 155.4 $\pm$ 1.4 Fg/l atrazine on Day 1; 17.5 $\pm$ 1.2 and 135.0 $\pm$ 4.5 Fg/L on Day 28 Interaction test with alachlor discussed under the section on pesticide interactions.	15.2 Fg/L (initial atrazine concentration): o 45% red. in benthic algal biovolume after 1 week sign. ($p \leq 0.05$); o 35% red. in benthic algal biovolume after 2 weeks non. sign. ($p \leq 0.05$); o 45% red. in benthic algal biovolume after 4 weeks sign. ($p \leq 0.05$). 155.6 Fg/L (initial atrazine concentration): o 45% red. in benthic algal biovolume after 1 week sign. ($p \leq 0.05$) o 50% red. in benthic algal biovolume after 2 weeks sign. ($p \leq 0.05$); o 57% red. in benthic algal biovolume after 4 weeks sign. ($p \leq 0.05$). Time-dependent analyses showed sign. ($p = 0.0083$) reduction in algal biovolume treated with both 15.2 and 155.6 Fg/L atrazine throughout the test, but no sign. ($p = 0.3629$) difference between 15.2 and 155.6 Fg/L levels.	A benthic mud community of epipelic algae were collected from various locations of Wahoo Creek and acclimated for 6 weeks prior to atrazine treatments. Stream water came from Wahoo Creek on March 25, 1993. Wahoo Creek is a third-order, sediment-dominated Nebraska stream draining primarily agricultural land and subject to major runoff events. Each model stream was constructed from a 114-L oval-shaped plastic tub and lined with two-layers of 4-mil clear plastic. Stream velocities ranged from 0.05 to 0.1 m/sec. in the sending segment and 0.01 to 0.05 m/sec. in the returning segment. Lighting was 12 hour/12 hour light/dark cycle. To replace evaporated water, stream water from the transport tank was mixed for 24 hours prior addition to each stream. Epipelic algae were sampled immediately before herbicide atrazine addition, 24 hours after addition, and after 1, 2 and 4 weeks. Algal samples were analyzed for cell density, cell biovolume and the relative abundance of 6 dominant taxa.	45020002 Carder & Hoagland 1998

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Natural Tasmanian stream: (2 weeks to 7 months: measured concentrations) Forests aerially sprayed once at either 3 or 6 liters ai per hectare of Gesaprim: peak of 22 ppb; median conc. of 2.5 ppb for the 2 weeks after application	Atrazine levels in 24 Tasmanian streams averaged 2.85 Fg/L (range< 0.01-53 mg/L). In forestry areas, the mean stream conc. was 2.00 (<0.01-8.9) Fg/L with 35% below the detection limit of 1.0 Fg/L. Spray drift into the stream appeared the same as in the treated forest as estimated by spray-droplet deposits on wood. 22 Fg/L: o sign. increase ($p < 0.01$) in daytime invertebrate drift at site 2, 12 hours after treatment o site 3 also showed an increase in daytime invertebrate drift on day of treatment, but not statistically sign. ($p > 0.05$) o sign. ($p<0.001$) increase in night drift in number of hydroptylid larvae on days 1, 2, 4, and 9 o sign. ($p<0.001$) increase in night drift in number of hydropsychid larvae on days 2, 4, and 9 The effects of invertebrate drift at site 2 were associated with increased spray drift, during the 12 hours immediately following application. Poor habitat and limited taxa at site 2 precluded drift analyses on specific taxa. o no sign. affect on mean densities of benthic invertebrates, number of taxa or taxa proportions o 71% sign. ($p<0.01$) increase in trout population at site 2 sustained over four months o no sign. effect on fish mortality or physiology	Tasmanian stream, Big Creek, with a catchment area of 36 km^2 was studied for atrazine aerially sprayed on two forest areas of 20 and 66 hectares, at rates of 3 and 6 kg ai/ha on 13 and 14 October 1987, respectively. Three sampling sites were picked: Site 1 above the 2 plantations, sites 2 and 3 were just below each plantation. Each site consisted of an upstream riffle for invertebrate samples and an area 100 m downstream for sampling brown trout (*Salmo trutta*). Atrazine levels in 174 water samples from 44 sites from 24 streams averaged 2.85 Fg/L (range< 0.01-53 mg/L). Only 9.6% of samples were below detection limit (0.1Fg/L) and only 24 % were below 1.0 Fg/L. In forestry areas, the mean stream conc. was 2.00 Fg/L (range <0.01-8.9 Fg/L) with 35% below the detection limit of 1.0 Fg/L. The initial measured concentration in Big creek was 22 Fg/L, 2 weeks later atrazine averaged 2.5 (range 1.2-4.6) Fg/L, and over the following 2 months ranged from 0.01 to 0.09 Fg/L. Atrazine levels in a small seepage draining the 2 plantations range 0.8- 68 Fg/L over the next 2 months. Site 2 sediments ranged from 1.6 to 22 Fg/kg wet weight two weeks after spraying. No fish mortality or behavioral changes were recorded during applications. However, brown trout movement within the application area was significantly different (increased) than the upstream control movement. No changes in trout physiology were observed.	45020003 Davies *et al*., 1994 (Species are not native to North America; Raw data unavailable for statistical analyses)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Artificial stream in laboratory Technical Atrazine: 98.2% Experiment 1: Constant 12-day exposures at 0, 24 & 134 Fg/L atrazine Experiment 2 involved pulsed exposures of 4 herbicides mixed together at nominal concentrations of: Atrazine at 135 Fg/L; Alachlor at 90 Fg/L; Metolachlor at 200 Fg/L; Metribuzin at 20 Fg/L. Full concentrations on Days 8 & 9, halved on Days 10 & 11, and discontinued on Day 12.	Constant 12-day exposure tests (Days 8-17) 10 and 25EC: o 24 Fg/L: - 24% red. sign. (p<.001) in ash-free dry wt. at 25EC - 30% red. sign. (p<.01) in chlorophyll a at 25EC o 134 ug/L: - 47% red. sign. (p<.001) in ash-free dry wt. at 10EC - 31% red. sign. (p<.001) in ash-free dry wt. at 25EC - 44% red. s ign. (P<.001) in chlorophyll a at 25EC - 30% red. s ign. (P<.01) in chlorophyll a at 10EC Nutrient uptake was affected more by the 15EC difference, than the atrazine concentrations. Raw data were absent and statistically analyses could not be assessed. As cited: - 35% red. N uptake at 134 Fg/L at 10EC; not sign. - 25% red. N uptake at 134 Fg/L at 25EC; not sign. - 31% red. silica uptake at 134 Fg/L at 10EC; not sign. - 58% red. silica uptake at 134 Fg/L at 25EC; not sign. - 14% red. P uptake at 134 Fg/L at 10EC; not sign. - 8 % red. P uptake at 134 Fg/L at 25EC; not sign.	Six artificial streams consisting of a 7.5 cm OD x 123 cm long Pyrex glass tube were tested concurrently for pesticide effects on *aufwuchs* productivity and nutrient uptake (NO_2, NO_3, phosphorus PO_4 and silica were tested after an 7-day colonization period with natural waters from a third order stream in the Sandusky Basin, Ohio. Two experimental designs (continuous and pulsed exposures) were tested under constant lighting, flow rates of 7.8 mL/min. natural creek water and 1.0 mL/min. nutrient water for 20-day periods. Experiment 1. Two "streams" were exposed to continuous nominal atrazine concentrations of 0, 50 and 200 Fg/L at 25EC and then repeated at 10EC on Days 8-17. Experiment 2. Three streams were treated to pulsed exposures of a mixture of four herbicides. These results are not relevant to the risk assessment for atrazine.	45020007 Krieger, Baker and Kramer 1988 Supplemental (The solvent methanol 0.00057% v/v was not added to controls; raw data unavailable for statistical analyses)

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Two artificial model streams in laboratory continuously exposed for 30 days with 60-day recovery period and repeated 4 times in one year. Nominal concentration of 25 Fg/L technical grade atrazine dissolved in DMSO; atrazine concentrations in streams were not measured.	25 Fg/L Atrazine: After one year of 4 treatment and recovery cycles, it was reported that the treatment did not have any significant or lasting effect on macroinvertebrate population structure, periphyton standing biomass or rates of primary production and community respiration. Two out of 200 statistical tests showed significant effects for atrazine treatment: equitability ($p < 0.029$) during Winter , month 3, and taxa/sample ($P < 0.001$) during the Spring, month 3. Macroinvertebrate drift in streams increased abruptly upon injection in both controls and treatments which was attributed to the solvent rather than to atrazine. Initial drift samples were collected only in the autumn and summer. Drift in the summer samples were "substantially higher" in the atrazine-treated streams than in the DMSO-treated control. Pulses in the number of drifting organisms following toxicant/solvent injection were primarily due to *Baetis* mayflies.	Continuous-flow stream treatment for 30 days at 25 ppb, followed by 60 days of no treatment, and repeated 4 times for one year in artificial, 3.96 m.-long concrete-lined streams inside a laboratory. Invertebrate populations were introduced by colonization from incoming drift with water flowing from a natural creek over a one year period before treatment. Atrazine was injected into the flowing water for periods as described above. Benthic invertebrate populations as follows: two samples (10.2-cm diameter cores) during pretreatment were collected at 45-day intervals for 1 year. Three post-treatment samples were made every 30 days. 24-Hour invertebrate drift samples were collected were collected on days 1, 5, 10, 20, and 29 during treatment and on days 14, 42 and 60 during recovery periods. Dry and ash weights of periphyton standing crop on four 25 x 75 mm glass slides were sampled at 4-day intervals for 28 days before and after each treatment. 24-Hour gross primary production and community respiration rates (O_2 levels) were measured during the autumn on days 2, 4, 8, 15, 24 and 29 after treatment and on days 20, 42, 54 and 60 during the recovery period.	45020009 Lynch *et al*., 1985 Supplemental DMSO is not an acceptable solvent, because it accelerates the movement of chemicals across cell membranes. As such it represents a worst case exposure. Raw data were not available for statistical analyses. Three or four samples are considered inadequate for field samples to show anything short of severe effects.
Artificial model streams in laboratory: (7 days; nominal) Single applications to spring water; Brazos, Texas. Nominal test concentrations: 0, 100, 1000 and 10,000 Fg/L	o statistically significant reductions (*) in net stream community productivity compared to controls: Day 1 Day 3 Day 7 100 Fg/L 736 %* 117 %* 34 % 1000 Fg/L 1367 %* 227 %* 119 %* 10,000 Fg/L 1716 %* 264 %* 135 o sign. ($p<0.02$) increase in *Nitzschia* cell numbers o no significant effect on other dominant algal groups o no significant effect on community respiration rates o no significant effect on conductivity or alkalinity	Four replicate recirculating artificial streams per treatment. Each stream (2.43 m long, 12.5 cm wide and 6 cm deep) was lined with polyethylene plastic and a single layer of gravel. Water from Minter Spring is a nearly anoxic and has a constant temperature (21EC). The flow rate was about 5 cm/sec. The principal algae genera were *Anabaena, Nitzschia, Rhopalodia* and *Navicula*. Five weeks for colonization of benthic algae on glass slides. Each stream received a single treatment which was recirculated. Nominal conc. were 0, 0.1, 1.0 and 10 Fg/L. Endpoints were net community productivity, respiration rate, cell numbers of dominant species, conductivity and alkalinity.	45020010 Moorhead and Kosinski 1986 Supplemental (raw data unavailable)
Not assayed, nominal conc. of 5, 25, and 125 ppb	Snail (*Lymnaea palustris*)	Snails exposed to one time dosing in mesocosm of either 5, 25, or 125 ppb and monitored for 12 weeks, no affect on growth, fecundity, or saccharide metabolism.	45020013 Baturo *et al*., 1995

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected species and life stage	Narrative of Study Trends	MRID No. Author/Year
Mean concentrations over two months of 5, 10, 22, 68, 182, and 318 ppb	Phyto- and zooplankton	Mesocosms in Bavaria were treated with atrazine 3 times over 3 summer months. Dose responsive reductions in dissolved oxygen and pH were noted at concentrations greater than 5 ppb. Substantial biological effects were generally noted at concentrations ≥182 ppb. Some effects on copepod nauplii were noted at 68 ppb. Diatoms appeared to become the dominant phytoplankton.	45020022 Jüttner *et al*., 1995 Supplemental (raw data unavailable)

Table 1 - cont. Atrazine Field or Mesocosm Studies.

Application rate (lb ai/A) and/or Nominal/Measured Concentration	Affected Species and Life Stage	Percentage Affected in Comparison to the Control and Type of Effect	Author/ Year

Table 1 - cont. Atrazine Field or Mesocosm Studies.

Application rate (lb ai/A) and/or Nominal/Measured Concentration	Affected Species and Life Stage	Percentage Affected in Comparison to the Control and Type of Effect	Author/ Year
Measured and reported to decrease at a rate of 0.2 ppb per day after addition at nominal conc. of 20, 100, or 500 ppb	Phyto-plankton, aquatic plants, and aquatic insects	Turbidity was increased by atrazine presence (due to wind mixing and loss of macrophytes). Study results were similar to DeNoyelles *et al*., in that there was a dose responsive depression of phytoplankton growth, followed by an increase in biomass and numbers of resistant species. Macrophyte numbers declined except *Chara* sp. at $\geq$100 ppb concentrations. For benthic emerging insects, individual herbivorous species and groups were significantly reduced at all dose levels. Emerging insects were significantly reduced in number and richness at 20 ppb. Early emergence of some of the herbivorous insects was also noted.	45227706 Dewey, 1986

Table 1 - cont. Atrazine Field or Mesocosm Studies.

Application rate (lb ai/A) and/or Nominal/Measured Concentration	Affected Species and Life Stage	Percentage Affected in Comparison to the Control and Type of Effect	Author/ Year
Nominal concentrations of 20, 100, 200, and 500 ppb. Measurements bi-weekly or monthly but results based on nominal concentration	Phytoplankton	Results of single species assays, microcosm, and pond studies were compared. Carbon fixation was used as the end-point for all three study types. Laboratory results with eight algal species ranged from 37 to 308 ppb for carbon uptake inhibition EC_{50} values. Microcosm EC_{50} values ranged from 103 to 159 ppb. The mean pond EC_{50} was 100 ppb for carbon uptake and 82 ppb for chlorophyll-a inhibition. Authors stated that multiple laboratory studies or a microcosm study represent(s) entire ecosystem functional effects.	45020015 Larsen *et al*., 1986 Supplemental (raw data unavailable)

Table 1 - cont. Atrazine Field or Mesocosm Studies.

Application rate (lb ai/A) and/or Nominal/Measured Concentration	Affected Species and Life Stage	Percentage Affected in Comparison to the Control and Type of Effect	Author/ Year
Measured = nominal (50 ppb) at time zero, declined to 40% of nominal after 8 weeks	Aquatic plants and fish	Atrazine and esfenvalerate were applied together in mesocosms to examine possible synergism (reduction of macrophytes leading to extension of insecticide residues and increased fish mortality). Combinations of 50 ppb atrazine and esfenvalerate at 0.25 to 1.71 ppb did not result in synergism. However, *Chara* sp. totally replaced the co-dominant *Naja* sp. six weeks after application.	Fairchild *et al*., 1994
Day 1 measured concentrations of 80, 140, or 1,560 ppb	Periphyton	Applications were made to *in situ* limnocorrals in June (140 and 1,560 ppb) or June & July (80 ppb) and colonized periphyton slides were submersed in August and monitored for either 56 days (140 and 1,560 ppb) or 210 days (80 ppb). Trends from both years included a shift from a chlorophyte to a diatom community, and a development of some atrazine "resistant" colonies. Community production was reduced by 21% and 82% at the 140 and 1,560 ppb levels, respectively, and certain algae were reduced up to 93%. All biotic measures indicated reduced growth, with cell densities lagging productivity. All parameters except species richness returned to control levels prior to 56 days after first or second applications.	45020020 Hamilton *et al*., 1987 Supplemental (raw data unavailable)
Day 1 measured concentration of 80 ppb (two applications of 100 ppb made 35-days apart)	Phyto- and zooplankton	Elaboration of the 80 ppb treatment from Hamilton *et al*., 1987. Two weeks after first application, significant declines in multiple species of green algae were observed, whereas crypto- and dinoflagellates either increased or stayed the same. Low population densities persisted for 114 days after the second application. Average of . 25% fewer species in atrazine limnocorrals. Control and treated values equilibrated within one year of treatment. Only two zooplankters were affected (after the second application). A MATC was suggested to be between 100 and 200 ppb.	Hamilton *et al*., 1988

Table 1 - cont. Atrazine Field or Mesocosm Studies.

Application rate (lb ai/A) and/or Nominal/Measured Concentration	Affected Species and Life Stage	Percentage Affected in Comparison to the Control and Type of Effect	Author/ Year
Measured after a single dose at 1,100 ppb - Day 1: 200 ppb, 55 days later: 60 ppb	Phytoplankton	Treatment related reductions in oxygen, and pH, and increases in conductivity were noted after atrazine treatment, with oxygen and pH returning to control values within 30-40 days. At 26 days after dosing, 78 algal cells/mL were present in the control and no cells were present in the treated enclosures. Diversity was also reduced the month after application.	45020016 Lay *et al*., 1984 Supplemental (raw data unavailable)
Not assayed, nominal concentrations of 50,000, 100,000, and 150,000 ppb	Autotrophs	Primary production and respiration was monitored in a freshwater ecosystem in India. Net productivity in water samples was reduced by 23% and 73%, respectively, at 50,000 and 100,000 ppb, in comparison to control values, and was negative in the 150,000 ppb treatment group.	Piska and Waghray, 1990

Table 1 - cont. Atrazine Field or Mesocosm Studies.

Application rate (lb ai/A) and/or Nominal/Measured Concentration	Affected Species and Life Stage	Percentage Affected in Comparison to the Control and Type of Effect	Author/ Year
Nominal applications of 0.4, 4.5, or 45 lb ai/A	Salt marsh edaphic alage	Elaboration of Plumley *et al*., concerning the carbon uptake for algae in the top 0.5 cm of enclosure sediment. Carbon fixation was significantly reduced at the 0.45 and 4.5 lb ai/A treatment levels for 16 days and at the 45 lb ai/A treatment level for 42 days.	45087406 Plumley and Davis, 1980

c. Marine and Estuarine Waters

Application rate (lb ai/A) Nominal/Measured Conc.	Concentration affecting endpoint (time to effect) o Affected Species and Life Stage	Narrative of Study Trends	MRID No. Author/Year
Marine Mesocosm: Open Ocean: Phytoplankton: (15 days; measured conc.) Measured = nominal at time zero, concentrations of 0.12, 0.56, and 5.8 ppb	0.12 ppb (differences compared to controls) o sign. lower pH levels (Days 5-14); indicative of reduced photosynthesis o higher dissolved organic nitrogen (DON) (Days 6-11) o up to 50% red. primary production (Days 3-11) o up to 60% red. particulate carbohydrates (Days 5-15) o up to 70% red. chlorophyll (Days 4-15) 0.56 ppb o sign. lower pH levels (Days 5-13) o incr. total dis. organic phosphate (DOP) (Days 3-14) o higher DON (Days 5-15) o up to 50 % red. primary production (Day 3-13) o up to 85% red. particulate carbohydrate (Days 5-15) o up to 80% red. chlorophyll (Days 4-15) 5.8 ppb o sign. lower pH levels (Days 5-11) o up to 200% increase in total DOP (Days 3-14) o up to 200 % increase in total DON (Days 2-15) o up to 50% red. in primary productivity (Days 3-7) o up to 60% red. in partic. carbohydrates (Days 5-15) o up to 30% red. in chlorophyll conc. (Days 4-15)	Mesocosms (2 m^2) inoculated with the diatoms *Thalassiosira punctigera*, *T. rotula*, *Nitzschia pungens* and *Skeletonema costatum* and a prymnesiophtye, *Phaeocystis globosa*. evidenced a dose-responsive elevation in dissolved nitrogen and phosphorous and reduction in primary production at 0.12, 0.56, and 5.8 ppb. The NOEL was reported to be <0.12 ppb. Atrazine at concentrations at 0.12, 0.56 and 5.8 ppb, adversely effects primary production of unicellar algal species at certain growth phases and causes increases in "excretions" of dissolved organic nitrogen and phosphorus. "Excretions" may be caused by atrazine stress on cells or lysis of cells.	45020021 Bester *et al*., 1995 Supplemental (raw data unavailable)

f. Reported Ecological Incidents

The Ecological Incident Information System (EIIS) maintained by EFED has a total of 109 reported incidents, as of November 26, 2000. The incidents occurred between 1991 and 1999 are of varying certainty for atrazine. Thirteen incidents were classified as "Unlikely" and two were "Unrelated." In only one case, a 1996 cotton incident, were fish carcasses analyzed for atrazine residues. The shad and carp tested positive for atrazine in the Richland, Louisiana incident (I004021-004), but the incident was determined as unlikely that atrazine was the causal factor. Fish kills (33 incidents) were the most frequent type of incident. Other non-target organisms affected include grasses, corn and on occasion: fruit trees, ornamentals, garden, raspberry, oats, cats, chicken, a goat, black snake and a cave amphipod.

Four incidents are listed as "Highly Probable" including a home/lawn use incident (# I001910) and a corn use incident affecting 100 bass and 100 bream (# B000163-001) resulting from registered use. Two home/lawn incidents affecting grass were concluded to be misuse/accidental (#I005132-001, I005579-001, I009445-031). Seventeen incidents are listed as "Probable" including

7 corn incidents (I007372-002, all bluegill and largemouth bass; I000116-002, thousand bluegill and thousand largemouth bass, I001081-001, 10 feet of grass and 600 catfish; I001081-002, bluegill and bass; B000150-003, bass and bluegill; I004697-084, fish; I000636-032, bluegill and a few crappie);

4 agricultural use incidents (affected grasses: I001081-001, I001081-002, I001099-001, grasses; I001041-001, fescue grass; I003826-006, not reported; I005895-074, not reported);

1 home/lawn incident (grasses: I000941-078, I008027-005, I008027-006, I008027-007, I008571-030L, I009445-030, I009445-033, I009445-034), 1 field incident (I005595-001, unknown) and 1 unreported source (I000358-004, fruit trees and garden). Two probable incidents were classified as misuse from corn use (I005879-003, pears, raspberry and oats; I007371-013, grass and ornamentals).

Twenty-six incidents were reported as "Possible" including 12 incidents affecting fish (i.e., bluegill (3 incidents), catfish (2), bass (2), carp (1), quillback carpsucker (1), carp (1); redhorse (1), bream (1), garfish (1), minnow (1), perch (1), unspecified fish (2); cave amphipod (1), corn (4), grass (4), trees (2), plants (1), cat (1), chicken (1), goat (1), and unreported (3).

Given the low toxicity of atrazine to fish, aquatic invertebrates and mammals, the reason for the frequency of effects on these organisms is uncertain. About 60 percent of the reported fish kills listed under atrazine in the incident record occur during the Spring when atrazine is applied, soils are saturated and heavy rainfall is frequent. Heavy runoff may carry atrazine, other pesticides and organic loads into surface waters. The high volume and wide-spread use of atrazine increases the probability of co-occurrence of fish kills with atrazine applications. There are some scenarios which may explain atrazine induced fish kills as well as causes unrelated to atrazine use.

Three plausible scenarios could exist in which atrazine applications may be responsible for the fish kills. First, atrazine concentrations in surface waters from runoff and/or spray drift may be much higher in shallow

water adjacent to treated fields than estimated by EFED or found in monitoring studies. Second, atrazine in surface water may kill aquatic plants and the decaying process of dead plants may lower dissolved oxygen to levels too low for fish survival. Third, a number of authors have reported that atrazine increases the toxicity of organophosphate insecticides, such as chlorpyrifos, and a number of other pesticides which may have been applied earlier to atrazine-treated crops or applied in other fields upstream in the watershed. See the section on Pesticide Toxicity Interactions below.

Possibilities also exist that other causes, not atrazine, may be responsible for some or all of the reported atrazine incidents. Heavy organic loads consume oxygen from the water as the organic matter oxidizes, thereby causing low dissolved oxygen levels which may cause fish to suffocate and die. Other pesticides in the watershed killed the fish as the water flowed past atrazine-treated fields. Since limited information is available in the atrazine incident records, such as water and tissue analyses, conclusions of responsibility would appear to be uncertain and the result of coincidence with little evidence for cause and effect.

g. Effects of Environmental Factors and Life-Stage on Aquatic Atrazine Toxicity

1. Interaction Effects on Atrazine Toxicity to Plants

Some intra-laboratory studies suggest that atrazine toxicity is affected by some environmental parameters, such as temperature, light intensity and salinity levels. Mayer *et al*. (1998) concluded that a temperature difference of 1EC will cause a difference in algal growth rate in the range of 7 to 9 percent at the typical rate increase for 10EC temperature increase (Q_{10}) of 2 to 2.3.

In general, the toxicity of pesticides increase with increasing temperature. Mayasich, Karlander and Terlizzi, Jr. (1986) tested two algal species in 27 combinations of temperature (15, 20 and 25EC), light intensity (0.208, 0.780 and 1.352 mW/cm^2) and atrazine concentrations of 0, 50 and 100 Fg/L) for 7-day periods. Toxic effects of atrazine on *Nannochloris oculata* growth rates were significantly ($p \leq 0.01$) dependent on both temperature and light intensity as determined by the 3-way interactions. Atrazine toxicity increased to *N. oculata* with both increasing temperature and increasing light intensity, except at 15EC and 1.352 mW/cm^2 where growth was intermediate. Previous results yielded a similar anomaly and it suggest that 15EC is near the lower limit for growth of this algal species. With *Phaeodactylum tricornutum*, growth rates were significant ($p \leq 0.01$) for light intensity and atrazine concentrations and were significant ($p \leq 0.05$) for temperature, but only light intensity was significantly ($p \leq 0.01$) related to an increase in atrazine toxicity. Atrazine toxicity was highest at the lowest light intensity. "The response of *P. tricornutum* to atrazine at light intensities of 0.780 and 1.352 mW/cm^2 is probably a reflection of primary effects only while at 0.208 mW/cm^2 light intensity includes secondary effects" (Mayasich *et al*., 1986). With respect to the insignificant effect of temperature on growth, Ukeles (1961) and Fawley (1984) found that the growth of *P. tricornutum* was unchanged by temperatures in the range of 14 to 24EC and 14 to 25EC, respectively.

Mayasich *et al*. (1987) repeated the above algal study with lower atrazine concentrations (0, 15, 30 and 50 Fg/L and fewer temperatures (15 and 25EC) and light intensities (0.208 and 1.352 mW/cm^2) in unialgal and bialgal assemblages. Generally *Phaeodactylum tricornutum*'s presence significantly ($p \leq 0.01$) depressed the growth of *Nannochloris oculata*, but it did not alter the magnitude of the responses to temperature, light intensity or atrazine concentrations. In contrast, the presence of *N. oculata* generally resulted in significant

($p \leq 0.01$) enhancement of *P. tricornutum* growth. The bialgal assemblage produced magnitudes of interactions between temperature and light intensity and temperature and atrazine were both significantly ($p \leq 0.01$) greater for *N. oculata*. *P. tricornutum* dominated the assemblage over all concentrations of atrazine under simultaneously low levels of temperature (15EC) and light intensity (0.208 mW/cm^2). At simultaneous high levels of temperature and light intensity and the absence of atrazine, *P. tricornutum* and *N. oculata* tended to be co-dominant. At increased atrazine concentrations, *P. tricornutum* became the dominant of the two algal species. The authors concluded that the enhanced sensitivity of *N. oculata* to atrazine relative to that exhibited by *P. tricornutum* indicated that atrazine posed a threat to the diversity and structure of natural phytoplankton populations. Thus, *Nannochloris oculata*, a nutritious algal species, for larval oysters (Dupry, 1973) is replaced by what is considered to be a poor food source for larval bivalves (Walne, 1970).

Mayer *et al*. (1998) tested the effect of four main environmental factors on the toxicity of atrazine to the green alga *Selenastrum capricornutum* in 3 day tests. The four factors tested were light intensity (44 and 198 FE/m^2), temperature (16 and 26EC), nitrogen source (NH_4+ and NO_3-) and pH (7.6 and 8.6). Temperature influenced growth only indirectly by interacting with light intensity. Algal growth measured as the atrazine EC_{50} values was marginally reduced under low light intensity at high and low temperatures (158 and 159 Fg/L, respectively versus the atrazine control, 164 Fg/L). High light intensity at the low temperature reduced the toxicity of atrazine to the alga by about two fold (LC_{50} 300 Fg/L) while high light intensity and high temperature reduced the toxicity of the atrazine by about 118 fold (LC_{50} 191 Fg/L). Nitrogen source and pH had no significant effect on atrazine toxicity affecting algal growth rates.

The above studies indicate that the toxicity of atrazine to plants can be affected by environmental parameters, but differences in those effects occur depending on the algal species. Hence, increases in temperature may increase, decrease or have no effect on atrazine toxicity to algal growth. Light intensity generally has the stronger effect on atrazine toxicity to algal growth and may, short of the point of photo-inhibition, increase the toxicity of atrazine. Nitrogen source and pH do not have any effect on the toxicity of atrazine to algae.

2. Interaction Effects on Atrazine Toxicity to Aquatic Animals

Some intra-laboratory studies suggest that atrazine toxicity to aquatic animals is affected by environmental parameters, such as water hardness, salinity and differences in the life-stages of organisms.

High levels of water hardness usually reduce the toxicity of pesticides. Intra-laboratory studies on two fish species provide comparative LC_{50} values for two levels of water hardness (Birge, Black and Bruser, 1979). Embryo-larval rainbow trout were exposed to atrazine for 27 days at water hardness levels of 50 and 200 mg/L and produced LC_{50} values of 0.66 and 0.81 mg/L, respectively. The test with channel catfish at the same water hardness levels for 8 days and yielded LC50 values of 0.22 and 0.23 mg/L. With rainbow trout embryo-larvae, the soft water increased toxicity by about 19 percent, while the LC_{50} values for embryo-larval catfish were the same. It is uncertain, if the shorter exposure period, yolk sac or differences in species sensitivity, account for the difference in water hardness effects between embryo-larvae of channel catfish and rainbow trout.

Salinity effects at 5, 15 and 25 g/L on the toxicity of atrazine are opposite for the estuarine fish larvae, sheepshead minnow and the copepod nauplii, *Eurytemora affinis* (Ziegenfuss, Anderson, Spittler and Leichtweis, 1994). The 96-hour LC_{50} values (16.2, 2.3 and 2.0 mg/L) for sheepshead minnow consistently increased with increasing salinity. In the case of the copepod nauplii, the 96-hour LC_{50} values (i.e., 0.5, 2.6 and 13.3 mg/L) consistently decreased with increasing salinity. The consistency of the two data sets suggest that salinity effects the toxicity of atrazine. Statistical tests for both species indicate significant differences between the LC_{50} valves at 5 and 25 g/L, but not at 15 g/L. The authors concluded that the two species may be more physiologically effective in metabolizing and mitigating toxic effects of atrazine at various salinities. The increase in LC_{50} values for rainbow trout and sheepshead minnow are consistent for increasing water hardness and increasing salinity.

For many organisms, the earlier life-stages are normally more sensitive to pesticides than later life-stages. Contrary to most organisms, the aquatic toxicity data for toad and frog tadpoles suggest that the late stages are more sensitive to atrazine than early tadpole stages (Howe *et al*., 1998). The late stage of the American toad tadpole is about 2.5 times more sensitive to atrazine than the early stage (10.7 versus 26.5 mg/L). For the northern leopard frog tadpoles, the later stage is about 3.3 times more toxic than the early tadpole stage (14.5 versus 47.6 mg/L).

The above studies suggest that decreases in water hardness and salinity can increase the toxicity of atrazine to fish, but increasing salinity may mitigate atrazine toxicity to copepods. Life stages show differences in sensitivity to atrazine. The later stages in frog and toad tadpole development show an increased sensitivity to atrazine over early tadpole stages.

h. Pesticide Toxicity Interactions

1. Plants

A number of authors have reported toxic interactions between atrazine, its dealkylated degradates and other pesticides. Synergism between atrazine and a number of other pesticides has also been reported in aquatic organisms, particularly with organophosphate insecticides, a carbamate insecticide and other herbicides.

In 1974, Putnam and Penner reported on the effects of interactions of herbicides on higher plants. Atrazine was cited in test combinations with 5 herbicides, 2 insecticides and a fungicide. Synergistic effects (i.e., increased toxicity higher than additivity) were identified in 6 out of the 8 test combinations. Atrazine was synergistic with 4 herbicides (i.e., 2, 4-D (oil), paraquat, EPTC, and alachlor) and 2 insecticides (i.e., diazinon and fensulfothion). Atrazine test combinations with dalapon, a herbicide, and dexon, a fungicide, showed antagonistic interactions.

Torres and O'Flaherty (1976) report additive toxicity of atrazine with simazine at concentrations of 1.0 Fg/L and 1,000 Fg/L for *Chlorella vulgaris*, *Stigeoclonium tenue*, *Tribonema* sp., *Vaucheria geminata*, and *Oscillatoria lutea*. Additive toxicity of malathion with atrazine was found in *Chlorella vulgaris*, but could not be assessed with other species, because malathion produced total inhibition of chlorophyll production at 1 Fg/L or greater concentrations. At 1 and 1,000 Fg/L, pesticide mixtures increased toxicity from 2.4 to 100

percent over the toxic levels of atrazine alone. Mixtures of these pesticides at concentrations of 0.1 and 0.5 Fg/L usually enhanced the production of chlorophyll.

Stratton (1984) also tested the most sensitive algal species, *Anabaena inaequalis*, with mixtures of atrazine and its two most toxic degradates, deethylatrazine and deisopropylatrazine. Cell count results indicate that combinations of atrazine/deethylatrazine (1.8) and atrazine/deisopropylatrazine (1.3) are synergistic and deethylatrazine/deisopropyl-atrazine mixtures are additive (1.03). For photosynthesis, results after 3 hour exposures indicate that all mixture combinations for these three chemicals are antagonistic (0.8, 0.86, and 0.89).

Burrell *et al*. (1985) reported 11-day interactions between algal populations and between algal populations and pesticides. Population interactions showed that *Chlorella vulgaris* inhibited population growth of *Ankistrodesmus braunii* by 32 percent. The addition of the bacterium, *Chromobacterium violaceum*, added to the algal mixture further inhibited population growth of *A*. *braunii* by an additional 17% and bacterial growth was stimulated, but the bacterium had no effect on *Chlorella* populations. The combined effect of the mixtures of atrazine (60 Fg/L) and sodium pentachlorophenate (Na-PCP) (0, 300, 800, 1,000 and 1,200 Fg/L) and atrazine (40 and 100 Fg/L) with Na-PCP (700 and 1,200 Fg/L) on *A*. *braunii* populationsF were additive over a wide range of concentrations. Similar results of atrazine (10 and 100 Fg/L) and Na-PCP (300 and 1,200 Fg/L) were obtained with C. *vulgaris.* In mixed algal cultures tested with atrazine (40 and 100 Fg/L), cell numbers of *A*. *braunii* were reduced 50 and 80 percent, respectively, which was not significantly different than effects when tested alone. In the same mixed culture test, atrazine inhibited growth of C. *vulgaris* by 79 and 85 percent, respectively, which showed a significant growth inhibition only at the lower test concentration (40 Fg/L). The authors concluded that the high atrazine concentration (100 Fg/L) did not alter the established population relationship between the two algal species, but at the lower concentration (40 Fg/L), *A*. *braunii* increased the susceptibility of *C*. *vulgaris* to atrazine. When mixed cultures of algae were treated with both atrazine (60 Fg/L) and Na-PCP (300, 800, 1,000 and 1,200 Fg/L), chemical antagonism was observed. The addition of the bacterium, *C*. *violaceum*, to the microcosm, had no effect on the level of antagonism for *A*. *braunii*. *C*. *violaceum* modified the antagonism of atrazine toxicity to *C*. *vulgaris* by about 40 percent, but the antagonistic effect was not eliminated. The net atrazine toxicity decreased as the Na-PCP concentration increased. The authors found no reason for the modification of atrazine effects by *C*. *violaceum*.

Carder and Hoagland (1998) reported that pesticide interactions of atrazine (0, 12 and 150 Fg/L) and alachlor (0, 5, 90 Fg/L) on benthic algal communities in artificial recirculating streams showed significant interaction (i.e., antagonism) only the first week in the combination of high alachlor and low atrazine test concentrations. The authors concluded that the interaction is most likely anomalous and the lack of significant synergistic effects may be attributed to different modes of action.

2. Aquatic Animals

A number of authors have reported synergistic effects of atrazine with the aquatic animals with one or more of the following pesticides: (i.e., alachlor, chlorpyrifos, DDT, malathion, methyl parathion, parathion and trichlorfon).

Liang and Lichtenstein (1975) also found atrazine synergism between soil residues of both DDT and parathion using fruit flies, *Drosophila melanogaster* and measured lethal effects versus the age of the pesticide residues in soil. Ten grams of Plainfield sand (1.2 % organic matter) or Plano silt loam (4.7% organic matter) was mixed with parathion (2.3 Fg/10 g of soil = 0.23 ppm) or DDT (30 Fg/10 g of soil = 3 ppm), then was mixed with 10 g of the same soil type, which contained increasing atrazine levels (40 to 1000 Fg/10 g of soil = 4 to 100 ppm) or controls. Fifty fruit flies were placed in 120 ml test jars for 24 hours with the 10-g portions of air-dried soil untreated or treated with atrazine, parathion, DDT or combinations thereof. The resulting 24-hour fruit fly LD_{50} values for constant soil levels of parathion (2.3 ppm) and DDT (3.0 ppm) were as follows: parathion (6.2 ppm atrazine in sand and 92 ppm in loam) and DDT (8.5 ppm atrazine in sand and 68 ppm in loam). Synergistic effects were apparent in all test combinations of soil and pesticides yielding a dose-response effect on fly mortality with increasing atrazine soil concentrations. Fruit fly mortality levels with both parathion and DDT in soils also clearly indicate a strong reduction in toxicity with the silt loam soil with a higher percentage of organic matter (4.7%) compared to sandy soil (1.2%).

Additional loam soil toxicity tests were conducted daily for 4 days, with aged-atrazine soil with an initial 50 ppm aged in the dark at 22EC and both fresh and aging-parathion soil levels (0.35 ppm). In the test with fresh parathion soils and aged-atrazine soils, toxicity to fruit flies decreased linearly from 95% mortality on Day 0 to 43.3% over four days. By the fourth day, atrazine levels had declined to 19 ppm, which was sufficient to synergize parathion in loam soils. In another toxicity test, parathion-treated soils were aged under the same conditions as above and added daily to the initial 10 g of atrazine-treated soil (50 ppm). In this test, the toxicity to fruit flies decreased logarithmically from about 68% on Day 0 to 10% mortality on Day 4. The measured concentrations of aging parathion in the silt loam soil decreased at a rate paralleling the logarithmic toxicity curve. The final parathion level on Day 4 was 0.24 ppm.

Liang and Lichtenstein (1975) found atrazine to be synergistic with parathion in 24-hour aquatic tests with third-instar mosquito larvae, *Aeddes aegypti* and also assessed the effects of sand and loam soils on their individual and combined toxicity in 20 ml of pesticide-treated water. Atrazine at 10,000 Fg/L showed no toxicity to the mosquito larvae; alone, parathion (15 Fg/L) killed 20 $\pm$ 7 percent of the larvae; and at these concentrations, the combination of the two pesticides produced significantly ($p = 0.01$) higher mortality (73 $\pm$ 18 %). Addition of 5 g of Plainfield sand (1.2% organic matter) with 15 Fg/L parathion reduced the toxicity of parathion from 20 $\pm$ 7% to 18 $\pm$4%, but when sand was mixed into the water, mortality drop to 5%. Plano silt loam soil (4.7% organic matter) without mixing reduced parathion toxicity from 20 $\pm$ 7% to 5 $\pm$ 4% and when the loam soil was mixed into the water, no mosquito larvae died. When these two soils were added to the same combination of atrazine and parathion, sand reduced the mortality from 73 $\pm$ 14% to 71 $\pm$ 14% (unmixed) and to 18 $\pm$ 4 % when mixed into the water; loam soil reduced the mortality from 73% to 64 $\pm$ 4% (unmixed) and to no mortality with mixing. The combination of atrazine and parathion was significantly ($p = 0.01$) more toxic than the toxicity of parathion or atrazine alone.

The above toxicity test method was repeated using 1 and 5 grams of sand or silt loam to measure the effect of different amounts of soil on toxicity following 24-hour exposures. Atrazine (10 ppm) produced no mortality in 24 hours to mosquito larvae. Parathion (0.015 ppm) produced 24 $\pm$ 7% mortality (no soil), 16 $\pm$ 7% (1g of sand), 2 $\pm$ 2% (5 g of sand), 7 $\pm$ 0% (1 g of loam soil) and 0% (5 g of loam soil). The combination of atrazine (10 ppm) and parathion (0.015 ppm) showed synergistic effects on mosquito larvae mortality: 62 $\pm$ 8% (no soil), 42 $\pm$ 10% (1 g of sand), 2 $\pm$ 2% (5 g of sand), 22 $\pm$ 4% (1 g of loam soil) and 0% (5 g of loam

soil). This test procedure was repeated using higher pesticide concentrations and again the mortality levels were increased with a mixture of atrazine (20 ppm) and parathion (0.30), but the synergistic increase was much lower than in the previous test. The 24-hour results indicated that atrazine alone was not toxic to mosquito larvae. Parathion (0.30 ppm) caused 93 $\pm$ 6% mortality in the absence of sediments; mortality was reduced to 62 $\pm$ 8% with 5 g of sand; and no mortality occurred with silt loam soil. When mixtures of 20 ppm atrazine and 0.30 ppm parathion were shaken with and without sediments, the mixture produced 98 $\pm$ 4% mortality with no soil, mortality decreased to 76 $\pm$ 4% with 5 g of sand and decreased to 38 $\pm$ 10% with 5 g of silt loam soil. These studies demonstrate that atrazine is synergistic with parathion and, like single toxicants, organic matter in soils and sediments will modify toxicity of pesticide mixtures, especially if the organic matter is suspended in the water. While this particular study has limited value for risk assessment, because the atrazine levels (10 and 20 ppm) exceed the normal environmental range of atrazine exposures, the study suggests that synergism of atrazine and parathion may occur at lower concentrations, possibly in the range of environmental atrazine levels.

Pape-Lindstrom and Lydy (1997) tested atrazine with 6 pesticides for chemical interactions using 4th instar midges (*Chironomus tentans*). The 96-hour test results for the pesticide mixtures indicated that atrazine was synergistic with the phosphonate insecticide, trichlorfon, (0.26 toxic units) and 3 phosphorothioate insecticides (i.e., malathion (0.36 TU), chlorpyrifos (0.58 TU) and methyl parathion (0.59 TU). The atrazine-mevinophos (a phosphate) mixture was less than additive (1.34 TU), while methoxychlor, a organochlorine insecticide mixture was also less than additive (1.67 TU). The results from these tests are questionable, since DMSO was used as a solvent with atrazine. These tests were repeated by Belden and Lydy (2000) without DMSO and with lower atrazine concentrations (0, 10, 40, 80, and 200 Fg/L). Acute 96-hour tests with *Chironomus tentans* were conducted with each pesticide and EC_1, EC_5, EC_{15} and EC_{50} values were determined based on inability of the midge to swim when prodded with forceps. Chemical interactions were tested at each of these EC levels with atrazine levels of 0, 10, 40, 80 and 200 Fg/L using 5 replicates of 10 midges each. Atrazine increased the toxicity of chlorpyrifos, diazinon and parathion, but not malathion. The authors concluded that "Interaction terms were not significant for atrazine + methyl parathion and atrazine + diazinon; however, a significant interaction was found for the atrazine + chlorpyrifos test ($p = 0.002$, df - 12, $F = 2.94$)." Synergistic ratios were reported as follows: chlorpyrifos, 1.83 at 40 Fg/L and 4.00 at 200 Fg/L atrazine; at 200 Fg/L diazinon the SR was 2.71 and for methyl parathion, the SR was 1.94. The variety of chemical interactions produced by atrazine mixtures indicates that the effect of atrazine on an organism is dependent on the species, cocontaminant, and the concentration of atrazine. Additional tests with 200 Fg/L atrazine and chlorpyrifos showed that atrazine increased the uptake of chlorpyrifos by 42 percent, and that the atrazine induction of cytochrome-P450 increased the formation of the O-analog which increased the toxicity of chlorpyrifos at enviromentally relevant concentrations.

Howe *et al*. 1998 reported synergism between atrazine and alachlor, a herbicide, in tests with young rainbow trout, channel catfish and early and late tadpole stages of the northern frog and the American toad. The results are presented in the table below. (MRID # 45202910).

Species (stage)	Time (hour)	Atrazine LC50 (95% CI) mg/L	Alachlor LC50 (95% CI) mg/L	Atrazine-Alachlor LC50[a] (95% CI) mg/L	Additive Index[b] (95% CI)
Rainbow trout (0.8-1.0-gram juveniles)	24 96	31.6 (28.2 - 35.4) 20.5 (18.3 - 22.9)	10.6 (9.5 - 11.7) 9.1 (9.0 - 9.2)	9.5 (8.3 - 10.9) 6.5 (5.7 - 7.7)	-0.20 (-0.53-0.059) -0.03 (-0.28-0.15)
Channel catfish (0.9-1.1-gram juveniles)	24 96	51.3 (44.6 - 59.0) 23.8 (22.3 - 25.5)	23.8 (22.7 - 25.0) 16.7 (15.1 - 18.4)	11.1 (9.6 - 12.4) 7.5 (5.3 - 8.4)	0.29 (0.067-0.55)[c] 0.31 (0.072-0.57)[c]
Northern leopard frog (0.7-0.9-gr early larvae)	24 96	69.7 (63.1 - 77.2) 47.6 (41.4 - 54.8)	14.9 (13.3 - 16.6) 11.5 (10.1 - 13.2)	12.1 (11.0 - 12.9) 6.5 (5.7 - 7.7)	0.015 (-0.17-0.24) 0.43 (0.054-0.87)[c]
Northern leopard frog (1.4-1.9-gr late larvae)	24 96	45.3 (42.3 - 48.5) 14.5 (11.9 - 17.5)	7.3 (6.6 - 8.0) 3.5 (3.1 - 3.8)	5.9 (5.5 - 6.4) 2.1 (2.0 - 2.3)	0.07 (-0.12-0.25) 0.34 (0.069-0.56)[c]
American Toad (0.1-0.2-gr early larvae)	24 96	66.4 (58.9 - 74.9) 26.5 (23.0 - 30.5)	5.7 (4.7 - 5.8) 3.9 (3.7 - 4.2)	4.4 (4.2 - 4.6) 1.8 (1.7 - 1.9)	0.19 (-0.057-0.28) 0.89 (0.68 - 1.2)[c]
American Toad (0.4-0.5-gr late larvae)	24 96	15.8 (13.5 - 18.4) 10.7 (9.2 - 12.5)	4.3 (3.8 - 4.8) 3.3 (2.8 - 3.6)	2.9 (2.6 - 3.3) 1.5 (1.4 - 1.6)	0.17 (0.11 - 0.46)[c] 0.68 (0.34 - 1.0)[c]

[a] 50:50 mixture of atrazine 4L (40.8% ai.) and alachlor EC (43.0% ai.).
[b] An additive index greater than zero indicates greater than additive toxicity.
[c] Significant chemical synergy interaction between atrazine and alachlor.

h. US EPA, Office of Water, Water Quality Criteria

The Office of Water sets ambient aquatic life water quality criteria to be used under two sections of the Clean Water Act, section 304(a)(1) and section 303(c)(2). In section 304, the term represents a non-regulatory, scientific assessment of ecological effects. If water quality criteria associated with specific stream uses are adopted by a state as water quality standards under section 303, they become enforceable maximum acceptable pollutant concentrations in ambient waters within that state. Water quality criteria adopted in state water quality standards could have the same numerical values as criteria developed under section 304. However, in many situations states might want to adjust water quality criteria developed under section 304 to reflect local environmental conditions and human exposure patterns. Alternatively, states may use different data and assumptions than EPA in deriving numeric criteria that are scientifically defensible and protective of designated uses. It is not until their adoption as a part state water quality standards that criteria become regulatory.

The ambient aquatic life water quality criteria for atrazine are currently under review. An atrazine draft dated 1/10/01, is being published for public comment. The draft water quality criteria values for atrazine are presented in the table below.

OW, Draft Water Quality Criteria for Atrazine (Fg/L)		
	Criterion Maximum Concentration (CMC)	Criterion Continuous Concentration (CCC)
Freshwater Criteria	351.2	12.35
Saltwater Criteria	759.5	26.71

h. US EPA, OPP, Terrestrial and Aquatic Most Sensitive Toxicity Values

The most sensitive terrestrial and aquatic toxicity values used to assess risks from pesticide use are presented in the table below.

OPP, Terrestrial and Aquatic Toxicity Values for Atrazine			
	Acute Value	Dietary Value	Chronic Value
Terrestrial Organisms:	(mg/kg)	(ppm)	(ppm)
Birds	940	> 5,000 (30 % dead)	< 75
Mammals	224	—	10
Freshwater Organisms:	(Fg/L)		(Fg/L)
Fish	4,500	—	65
Invertebrates	720	—	60
Saltwater Organisms:	(Fg/L)		(Fg/L)
Fish	8,500	—	1,900
Invertebrates	88	—	80
Terrestrial Plants:	(lbs ai/A)		
Seedling Emergence:			
Dicot	0.003	—	—
Monocot	0.004	—	—
Vegetative Vigor;			
Dicot	0.008	—	—
Monocot	0.61	—	—
Aquatic Plants:	(Fg/L)		(Fg/L)
Freshwater Plants:			
Algae	< 1		25
Vascular Plants	2		2
Saltwater Plants:			
Algae	10		22
Vascular Plants	< 4		8

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